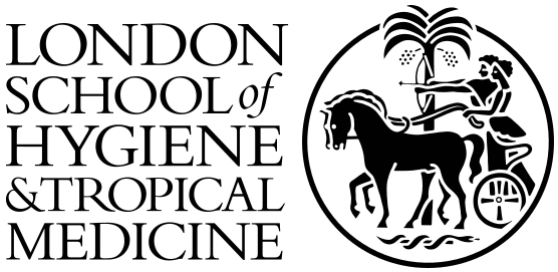

Impact of malaria in pregnancy and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants

ABEL KAKURU

Thesis submitted in fulfillment of requirements for the degree of Doctor of Philosophy

February 2021

Department of Clinical Research

Faculty of Infectious and Tropical Diseases

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

This work was funded through grants received from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (P01 HD059454), the Bill and Melinda Gates Foundation (OPP1141549), and the Fogarty International Center training grant (D43TW7375).

DECLARATION BY STUDENT

I Abel Kakuru, declare that this thesis is my own work. Where work of others has been referred to in this thesis, this has been indicated. This work has not been submitted previously for an academic qualification.

Name: Abel Kakuru

Date: February 2021

Signature

ABSTRACT

Background: Placental malaria (PM) has been associated with an increased risk of malaria during infancy in observational studies suggesting that effective intermittent preventive treatment of malaria in pregnancy (IPTp) may reduce the risk of malaria in infants. However, there are no randomised controlled trials that have shown that improved IPTp leads to less malaria during infancy. To address this knowledge gap, this thesis aimed to: 1) compare the incidence of malaria in infants during the first year of life among infants born to mothers with PM detected by histology and infants born to mothers without PM; 2) compare the incidence of malaria during the first year of life among infants born to mothers randomised to receive monthly IPTp with sulfadoxine-pyrimethamine (SP) versus those born to mothers randomised to receive monthly IPTp with dihydroartemisinin-piperaquine (DP); and 3) evaluate the effect of PM and IPTp on cord blood levels of IgG antibodies to *Plasmodium falciparum* malaria antigens in infants born to mothers enrolled in the trial.

Methods: Infants born to HIV-uninfected pregnant mothers who participated in a double-blind, randomised trial of monthly IPTp with SP or DP in Busia, Uganda were followed from birth to 12 months of age. The primary outcome was the incidence of malaria measured by passive surveillance during the first year of life. PM was categorised as: 1) no PM (no parasites or pigment), 2) active PM (presence of parasites), 3) mild-moderate past PM (>0-20% high powered fields [HPFs] with pigment), and 4) severe past PM (>20% HPFs with pigment). Cord blood IgG antibody levels to *P. falciparum* antigens: apical membrane antigen-1 (AMA1), erythrocyte binding antigen-140 (EBA140), EBA175, EBA181, glutamate-rich protein (GLURP), merozoite surface protein-1 (MSP1), reticulocyte-binding protein homologue-2 (Rh2), Rh4, and Rh5 were measured using a multiplex antibody bead assay.

Results: Between December 9, 2016 and December 7, 2017, 678 infants were born into the cohort, including 339 to mothers receiving IPTp-DP and 339 to mothers receiving IPTp-SP. A total of 581 infants (85.7%) were followed to 12 months of age. There were 1131 malaria episodes diagnosed in infants during follow-up. Compared to infants born to mothers with no PM, the incidence of malaria was higher among infants born to mothers with active PM (adjusted incidence rate ratio [aIRR] 1.30, 95% CI 1.00-1.71, p=0.05) and those born to mothers with severe past PM (aIRR 1.28, 95% CI 0.89-1.83, p=0.18), but the differences were not statistically significant. When the analysis was stratified by infant sex, the incidence of malaria was higher in male infants born to mothers with severe past PM than in those born to mothers with no PM (aIRR 2.17, 95% CI 1.45-3.25, p<0.001), but not in female infants (aIRR 0.74, 95% CI 0.46-1.20, p=0.22). The association between IPTp and malaria incidence in infants was modified by infant

sex. Compared to IPTp-SP, IPTp-DP was associated with a lower incidence of malaria among male infants (IRR 0·75, 95% CI 0·58-0·98, p=0·03), but not female infants (IRR 0.99, 95% CI 0.79-1.24, p=0.93). There was no significant difference in the cord blood levels of IgG antibodies to *Plasmodium falciparum* among infants born to mothers with active PM, mild-moderate past PM, or severe past PM compared to infants born to mothers with no PM, and among infants born to mothers who received IPTp-DP compared to those born to mothers who received IPTp-SP.

Conclusion: Severe past PM was associated with a higher incidence of malaria among male infants. IPTp-DP was associated with a lower incidence of malaria among male infants compared to IPTp-SP. PM and IPTp did not affect cord blood *P. falciparum* IgG antibody levels. These findings suggest that severe past PM may negatively impact antimalarial immunity in male infants and that highly effective IPTp may be protective among male infants.

ACKNOWLEDGEMENTS

First, I am grateful to God for the strength that he gave me to complete my PhD and for keeping my hope alive in a time of need.

Second, I am very grateful to my supervisors, Prof. Sarah G. Staedke, Prof Daniel Chandramohan, and Prof Grant Dorsey for the support and guidance you gave me through my PhD journey. You have been an amazing supervision team for me. I will always cherish you in my heart.

I would also like to thank my advisors; Dr Emily Webb for providing statistical support, and Prof Chris Drakeley for giving me guidance on the immunology-related portions. Special thanks to my upgrading examiners, Prof. Brian Greenwood, and Dr Matthew Chico for having accepted to examine me during the upgrading from MPhil to PhD. The guidance that you gave me during the upgrading was very vital. I am also grateful to Dr Prasanna Jagannathan and Dr Isaac Ssewanyana for giving me guidance on malaria immunology. Thank you, my fellow PhD students, especially Simon Peter Kigozi, Dr Swaib Lule, and Ms Julian Muwanguzi for making me feel at home at the London School of Hygiene and Tropical Medicine Campus.

I would like to thank the study participants who participated in this research. Special thanks to the IDRC team lead by Prof Moses R Kamya for the support they gave me. The Birth Cohort 3 study team for the job well done in collecting data. Special thanks also go to Prof Phil Rosenthal, and the Fogarty International Center team in Uganda, Deborah Ekusai, Dr Arthur Mpimbaza, for the support that they gave me during my studies. You made my studies smooth.

I am grateful to Ms Lydia Wendy Ndaruzi and her family for hosting me during my short stays in London. You provided me with a home away from home and made me feel at home. Your house always had plenty and was always warm, which I appreciated in those bad winter days of February 2018 when it snowed in London. You will always be my home in London. I would also like to thank the family of Mr and Mrs Karugaba Robin for welcoming me in the UK. Your warm home in Kent always provided me with a different environment from the fast life in London.

Lastly, I would like to thank my family, my lovely wife, Dr Mary Muhindo Kakuru, for the moral support that you gave me during my studies. You were there to keep the children while I was away and always praying for me. I am so grateful to you. I am also grateful to my children, Jemimah, Joel, Jeremiah, and Joanna, for enduring my absence from home during those days when I would travel to London. I cannot end without thanking my parents, Mr, and Mrs Polly Buhweire for giving me an opportunity by setting off my education path. I owe all this to you and may God bless you for being amazing parents.

.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

aHR	Adjusted Hazard ratio
AI	Adjusted for IPTp or insecticide treated net use
aIRR	Adjusted incidence rate ratio
AL	Artemether-lumefantrine
AMA-1	Apical membrane Antigen-1
AME	Adjusted for malaria transmission exposure
AO	Assessment of the outcome
AQ	Amodiaquine
AU	Arbitrary units
AZ	Azithromycin
CBC	Complete blood count
CBMC	Cord blood mono-nuclear cells
CF	Completeness of follow-up
CI	Confidence interval
CQ	Chloroquine
CSA	Chondroitin sulfate A
CSST	Community-scheduled screening and treatment
DNA	Deoxyribonucleic acid
DON	Demonstration that the outcome of interest was not present at the start of the study
DP	Dihydroartemesinin-piperaquine
EBA	Erythrocyte binding antigen
EDTA	Ethylenediaminetetraacetic acid
FL	Follow-up long enough for outcome to occur
GLURP	Glutamate rich protein
HIV	Human immunodeficiency virus
HPF	High-power fields
HR	Hazard ratio
ICAM	Intercellular adhesion molecule
IDRC	Infectious Diseases Research Collaboration
IFN	Interferon
IOW	Inverse odds weighting
IPTi	Intermittent preventive treatment of malaria in infancy
IPTp	Intermittent preventive treatment of malaria in pregnancy
IRR	Incidence rate ratio

IST	Intermittent screening and treatment
ITN	Insecticide treated net
LAMP	Loop mediated isothermal amplification
ME	Measurement of exposure to malaria during pregnancy
MeSH	Medical Subject Headings
MFI	Median fluorescence intensity
MiP	Malaria in pregnancy
MQ	Mefloquine
MSP-1	Merozoite surface protein-1
NICHD	National Institute of Child Health and Human Development
NOS	Newcastle Ottawa Scale
NR	Not reported
OR	Odds ratio
Pfdhfr	*Plasmodium falciparum* dihydrofolate reductase
Pfdhps	*Plasmodium falciparum* dihydropteroate synthase
PfEMP	*Plasmodium falciparum* erythrocyte membrane protein
PCR	Polymerase chain reaction
PM	Placental malaria
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
qPCR	Quantitative polymerase chain reaction
RCT	Randomised controlled trial
REC	Representativeness of the exposed cohort
Rh	Reticulocyte-binding protein homologue
RDT	Rapid diagnostic test
RR	Risk ratio
SP	Sulfadoxine-pyrimethamine
SNEC	Selection of the non-exposed cohort
TLR	Toll-like receptors
VAR	Variant surface antigen
WHO	World Health Organization

PREFACE

This thesis was written following the Research Paper Style guide in accordance with the London School of Hygiene and Tropical Medicine guidelines. The thesis consists of four research papers, two of which have been published, one has been submitted for consideration for publication and is currently under review, and the fourth is pending submission. All the published papers included in this thesis were published in open assess journals and copyright was retained by the authors.

CHAPTER 1 INTRODUCTION

1.1 Epidemiology and burden of *Plasmodium falciparum* malaria in pregnancy

Plasmodium falciparum malaria is a major global public health problem. In 2018, 228 million cases of malaria were estimated to have occurred globally, 96.7% of which were due to *P. falciparum* [1]. In sub-Saharan Africa, where most areas are endemic for *P. falciparum,* pregnant women and young children bear the greatest burden of malaria. Eleven million pregnant women were estimated to be at risk of *P. falciparum* infection in 2018 [1]. In areas of moderate to high malaria transmission intensity, young children are usually at higher risk of malaria than older children and adults because they have less immunity to malaria [2]. Immunity to *P. falciparum* is gradually acquired through repeated infection and exposure to parasites [3-5]. By adulthood, most residents of endemic areas have developed partial immunity such that *P. falciparum* infections are primarily asymptomatic.

P. falciparum infection, unlike other *Plasmodium* species that cause malaria in humans, is associated with severe disease in non-immune individuals mainly due to its unique ability to attach to microvascular endothelium leading to sequestration of parasites in body organs including lungs, brain, and placenta. *P. vivax* has been also shown to cause severe disease [6, 7] and is associated with adverse birth outcomes including low birth weight and preterm delivery [8, 9]. However, the risk of developing severe disease and adverse birth outcomes is lower with *P. vivax* infection than *P. falciparum* infection [10]. Pregnant women are at higher risk of *P. falciparum* infection than non-pregnant women. The reasons for this are not well understood, but hormonal and immunological changes during pregnancy [11, 12], and increased attractiveness of pregnant women to mosquitoes, have been suggested as possible explanations [13]. The course of *P. falciparum* infection in pregnant women depends on maternal antimalarial immunity, which is determined by malaria endemicity. In areas of low transmission intensity, pregnant women typically have little or no immunity to *P. falciparum* and susceptibility to infection is not dependent on maternal gravidity. *P. falciparum* infection in pregnant women with little antimalarial immunity is frequently symptomatic, severe, and is associated with a high risk of maternal mortality and foetal loss [14]. In areas of moderate or high malaria transmission intensity, pregnant women, like non-pregnant adults, have acquired partial immunity to malaria and primigravidae and secundigravidae women are more susceptible to *P. falciparum* infection than multigravidae women [15]. *P. falciparum* infection in partially immune pregnant women is frequently asymptomatic, but is associated with placental *P. falciparum* infection, a condition

known as placental malaria (PM), which is associated with maternal anaemia and adverse birth outcomes such as preterm delivery, low birth weight and stillbirths [16, 17]. In 2018, 11 million pregnant women were estimated to have been exposed to malaria in sub-Saharan Africa, and 872,000 infants suffered from low birth weight as a result [1].

In malaria endemic areas, primigravidae and secundigravidae women are more vulnerable to *P. falciparum* infection and its harmful effects because they lack antimalarial immunity specific to parasites that infect the placenta [18]. Immunity to *P. falciparum* is developed by acquisition of antibodies to parasite proteins important for attachment of parasites to host tissue receptors including chondroitin sulfate A (CSA) which are abundant in the placenta. Parasites isolated from placental specimens are immunologically distinct from those isolated from non-pregnant individuals, and predominantly express the variant surface antigen 2-CSA (*var*2CSA) gene, which encodes proteins for binding to placental CSA receptors [19, 20]. Immunity to malaria in pregnancy is developed by the acquisition of antibodies to CSA binding parasite proteins and primigravidae and secundigravidae women are at high risk because they lack these antibodies [18, 21]. Also, reduced cell-mediated immune responses have been suggested to play a role in the susceptibility of primigravidae and secundigravidae women to PM. This is supported by evidence of reduced cell-mediated immune responses in first pregnancies [22] which improve over subsequent pregnancies [23]. Together, the lack of antibody mediated immunity and the reduced cell-mediated immunity to PM, makes primigravidae and secundigravidae women at higher risk of PM and adverse birth outcomes than multigravidae women in malaria endemic areas.

1.2 Placental malaria and its associated pathological changes

P. falciparum expresses proteins on the membrane of infected erythrocytes which include *P. falciparum* erythrocyte membrane protein (PfEMP) 1, sequestrin, and histidine-rich protein 1 and 2 [24, 25]. These membrane proteins expressed on infected erythrocytes bind to host receptors including vascular cell adhesion molecule-1, intercellular adhesion molecule-1 (ICAM-1), platelet glycoprotein 4 (CD36), and CSA [24]. Of the *P. falciparum* membrane proteins expressed, PfEMP-1 is a key protein which binds a wide range of receptors including CD36, ICAM-1, and CD31 that have been implicated in the pathogenesis of both severe and uncomplicated malaria [26]. The binding of infected erythrocytes to the endothelium of microvasculature helps the parasites evade clearance by the spleen which removes infected erythrocytes [27, 28].

In pregnant women, the placenta is a preferred site for sequestration of *P. falciparum* infected erythrocytes. *P. falciparum* infected erythrocytes sequester in the placenta by binding to abundant CSA receptors [29] which leads to accumulation of parasites in the intervillous space

[30] even though parasites may not be detectable in the maternal peripheral blood [31]. *P. falciparum* isolates from the placenta have been shown to have a high affinity for binding to CSA receptors, but very low affinity for binding to CD36 and ICAM-1, the receptors commonly bound by PfEMP1 in non-pregnant individuals [20, 32] suggesting that parasites with high CSA binding affinity are selected for in pregnant women [33]. Sequestration of infected erythrocytes in the placenta leads to pathological changes including massive infiltration with inflammatory cells, presence of parasites, deposition of haemozoin (malaria pigment), a by-product of haemoglobin digestion by malaria parasites, and perivillous fibrin deposition [34], which are associated with maternal morbidity and adverse birth outcomes. Without treatment, some women can clear placental *P. falciparum* infection leaving only malaria pigment as evidence of past infection.

Diagnosis of PM is made at delivery by detection of parasites in placental blood or detection of pathological changes in placental tissue by histology. Detection of parasites in placental blood is done by microscopic exam of thick blood smears, rapid diagnostic tests [35], or by detecting parasite DNA using loop-mediated isothermal amplification (LAMP) [36, 37] or quantitative polymerase chain reaction (qPCR) [38]. Detection of PM by histology is done by microscopically examining placental tissue sections for the presence of parasites, inflammatory changes, and malaria pigment in fibrin or monocytes. Detection of parasites in placental blood by microscopy [39], rapid diagnostic tests or LAMP have lower sensitivity for the detection of PM [36, 37] than histology. Quantitative PCR has been suggested to have a higher sensitivity than placental histology [38], but fails to detect malaria pigment, a sign of past infection. Different methods, based on histological findings have been suggested for categorising PM including the Bulmer classification [40], the modified Bulmer classification suggested by Ismail et al [41], and the Rogerson criteria [42], which are summarised in table 1.1 In addition, a useful semiquantitative method for grading inflammation (presence of mononuclear cells graded qualitatively) and malaria pigment deposition in fibrin in intervillous space (proportion of high-power fields [HPF] with malaria pigment deposition in fibrin) has been suggested by Muehlenbachs et al [43].

Table 1.1: Classification of placental malaria based on histological findings

Placental malaria category	Description
Bulmer classification [40]	
Not infected	No parasites or pigment in monocytes or fibrin
Active infection	Parasites detected with pigment in monocytes but not in fibrin
Active chronic infection	Parasites detected with pigment in fibrin
Past chronic infection	No parasites, pigment in fibrin or cells within fibrin
Modified Bulmer classification [41]	
No infection	No parasites or malaria pigment
Active-acute	Presence of parasites with minimal or no pigment
Active chronic	Presence of parasites with considerable pigment
Past	Presence of pigment with no parasites
Rogerson criteria [42]	
Class 1	Presence of parasites without pigment in monocytes or fibrin
Class 2	Parasites present, pigment in monocytes ± fibrin
Class 3	Parasites present, pigment in fibrin
Class 4	Presence of pigment only without parasites (past infection)
Class 5	No parasites or pigment (No infection)

1.3 Association between placental malaria pathological changes and adverse birth outcomes

Different pathological findings associated with PM have been shown to be associated with maternal morbidity and adverse birth outcomes. The presence of inflammatory cells in the intervillous spaces has been associated with maternal anaemia [42] and low birth weight [42, 44]. The presence of infected erythrocytes and perivillous fibrin deposition was shown to be associated with preterm delivery [44], while presence of pigment deposition in monocytes and fibrin has been associated with low birth weight [42].

The exact mechanism by which PM leads to adverse birth outcomes is not well known, but several possible mechanisms have been suggested including maternal anaemia, inhibition of trophoblast invasion, and disruptions in maternal and placental hormones [45]. PM is thought to cause adverse birth outcomes through its association with maternal anaemia [46, 47] and maternal anaemia is independently associated with adverse birth outcomes including low birth weight and preterm birth [48]. Inhibition of trophoblast invasion by PM may lead to poor development of the placental vascular system leading to foetal growth restriction [49]. Disruptions in placental and maternal hormones have also been suggested as possible explanations for PM related foetal growth restriction. PM was reported to be associated with reduced levels of insulin-like growth hormone-1 in maternal and cord blood, which was found to be associated with low birth weight [50].

1.4 Effects of malaria in pregnancy on foetal immunity

There is mounting evidence that *P. falciparum* infection during pregnancy may affect foetal immune responses, which may alter risk of infectious diseases in the newborn later in life [51-55]. Evidence from laboratory studies shows that cord blood mononuclear cells (CBMCs) from newborns of mothers with peripheral malaria parasitaemia or PM have increased production of anti-inflammatory cytokines and reduced production of pro-inflammatory cytokines following stimulation with non-malaria specific antigens [56-58], and malaria specific antigens [56, 59-61], indicating a bias towards immune tolerance. In Gambia, following stimulation with *P. falciparum* antigens, CBMCs of infants born to mothers with placental parasitaemia had increased production of interleukin (IL)-10 and reduced production of interferon gamma (IFN-γ) compared to CBMCs of infants born to mothers without placental parasitaemia [56]. Fievet et al reported increased production of IL-10, following stimulation with synthetic haemozoin, in CBMCs from infants born to mothers with malaria pigment in placental tissue compared to those born to mothers without malaria pigment in the placenta [57]. In Benin, maternal *P. falciparum* infection at delivery (defined as parasites detected in placental or maternal blood) was associated with increased production of IL-10 following toll-like receptor (TLR)-3, 7/8 mediated stimulation of CBMCs with non-malaria specific antigens which was associated with increased risk of malaria during infancy [58]. In another study, γδ T- cells from CBMCs of infants born to mothers with malaria in pregnancy who were treated, demonstrated increased non-specific antigen-driven production of IFN-γ and tumour necrosis factor-α compared to CBMCs from infants born to untreated mothers [62] suggesting that treatment of malaria in pregnancy may improve immune responses in newborns. Together, these studies suggest that PM biases foetal immune responses towards tolerance to both malaria and non-malaria specific antigens, which may affect the infant's risk of infections later in life, and treatment of malaria in pregnancy may reduce this effect.

Malaria in pregnancy may also affect acquisition of antibodies by the newborn in-utero or later in life. Placental malaria has been associated with reduced maternal-foetal transfer of immunoglobulin G (IgG) antibodies to infectious diseases including herpes simplex type 1, respiratory syncytial virus and varicella zoster [63], tetanus [64, 65], measles [66], streptococcus [66] and *P. falciparum* malaria [67, 68] which may affect the risk to common infections during infancy [69]. Active PM has been associated with reduced maternal-foetal transfer of IgG antibodies to *P. falciparum* antigens including merozoite surface protein (MSP) 1-19, apical membrane antigen (AMA)-1 and glutamate rich protein [67]. But this reduction in transfer of antibodies to *P. falciparum* antigens was not found to be associated with increased risk of

malaria infection in the first 6 months of life [67]. In Kenya, PM was associated with reduced levels of IgG antibodies to *P. falciparum* antigens (circumsporozoite protein, MSP, rhoptry associated protein-1, and erythrocyte binding antigen-175) in infants 4-12 months of age [70] suggesting that PM may reduce acquisition of malaria-specific antibodies in infants. However, this study did not show a statistically significant association between the risk of malaria parasitaemia and reduced acquisition of malaria-specific antibodies, possibly due to small sample size. These studies suggest that PM may reduce maternal-foetal transfer of antibodies to *P. falciparum* and other non-malaria pathogens, but whether this affects the risk of malaria and non-malaria illnesses during infancy remains to be determined.

1.5 Association between malaria in pregnancy and malaria risk in infants

Several studies have reported an association between malaria in pregnancy and a higher risk of malaria in infancy. Infants born to mothers with PM detected by microscopy or histology were reported to have a higher risk of malaria, compared to those born to mothers without PM, in studies conducted in Malawi, Uganda, Benin, Gabon, Tanzania, and Cameroon [71-76]. However, these studies had two major limitations. First, in most studies PM was defined as detection of parasites in placental blood by microscopy which is less sensitive than placental histology [39] and is therefore likely to lead to misclassification and underestimation of the effect of PM on infant malaria risk. Secondly, most were observational studies and did not adjust for possible confounding by malaria transmission which is shared by both the mother and her infant [77]. One study, conducted in Benin, which did adjust for possible confounding by malaria transmission intensity, reported an increased risk of malaria in infants born to mothers with PM detected by microscopy compared to infants born to mothers without PM [73]. However, this effect was only observed in infants who slept in homes with mosquito bed nets and not in those who did not have bed nets. Further evaluation of the association between malaria in pregnancy and the risk of malaria in infants is needed, including randomised trials using highly efficacious interventions for prevention of malaria in pregnancy that significantly reduce malaria parasite burden in pregnancy and PM.

1.6 Prevention of malaria in pregnancy

To prevent placental malaria and improve birth outcomes for pregnant women residing in moderate to high malaria transmission intensity settings, the World Health Organization (WHO) recommends that all pregnant women sleep under an insecticide treated bed net (ITN). HIV-uninfected pregnant women should also take intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) starting as early as possible after the first

trimester and given at every antenatal visit at least a month apart [78]. Previously, IPTp-SP was shown to reduce the risk of maternal anaemia, PM, and low birth weight among primigravidae and multigravidae women [79-81]. However, currently, widespread antifolate parasite resistance [82] threatens the effectiveness of IPTp-SP necessitating alternative drugs and strategies for IPTp.

P. falciparum antifolate resistance develops by mutations in the parasite genes for two important enzymes in the parasite DNA synthesis: dihydrofolate reductase (*Pfdhfr*) and dihydropteroate synthase (*Pfdhps*). A combination of five gene mutations referred to as the quintuple mutations, consisting of three mutations in the *Pfdhfr* gene (N51I, C59R, S108N), and two mutations in the *Pfdhps* gene (A437G, G540E), has been associated with a higher risk of SP treatment failure in children [83] and reduced parasite clearance and failure to prevent new infections in pregnant women [84-86]. In most parts of East Africa, the prevalence of *Pfdhps* G540E, a proxy marker of the quintuple mutations has been reported to be over 50% [87]. But, despite the wide-spread quintuple mutations, a meta-analysis of data from studies conducted in sub-Saharan Africa showed that ≥3 doses of IPTp-SP compared to 2 doses, prevent low birth weight even in areas with high prevalence of quintuple mutations [88]. In West Africa, where in most areas the prevalence of quintuple mutations remains low [89], IPTp-SP remains effective at preventing low birth weight [90]. A sixth gene mutation in addition to the quintuple mutations, the *Pfdhps* A581G sextuple mutation, which is associated with complete resistance of parasites to SP [91] is emerging in East Africa [87]. In areas where prevalence of the sextuple gene is >10%, IPTp-SP has been reported to fail to prevent low birth weight [92] highlighting an urgent need for alternative drugs for IPTp.

Several studies have evaluated alternative drugs and strategies for IPTp, but these studies did not show superiority of the alternative regimens and some drugs were poorly tolerated. In Ghana, IPTp with amodiaquine (AQ) or IPTp with SP-AQ combination were not superior to IPTp-SP in preventing low birth weight and PM in HIV-uninfected pregnant women [93]. In addition, AQ-based regimens were associated with a higher risk of adverse events compared to IPTp-SP. Mefloquine (MQ) for IPTp in HIV-uninfected pregnant women was poorly tolerated and was not associated with a reduced risk of low birth weight or PM compared to IPTp-SP, despite association with a lower risk of malaria parasitaemia and clinical malaria during pregnancy [94]. A multicentre trial, conducted in Uganda, Tanzania, Malawi, Kenya, and Benin, of IPTp with fixed dose azithromycin-chloroquine (AZ-CQ) compared to IPTp-SP showed a slightly higher risk of adverse birth outcomes (abortion, preterm delivery, low birth weight) among mothers receiving IPTp-AZ-CQ and no difference in the risk of PM [95]. Furthermore, AZ-CQ was not well tolerated. This trial was deemed futile and was stopped early. Dihydroartemisinin-piperaquine (DP), a

highly effective artemisinin combination therapy, has also been evaluated for prevention of malaria in pregnancy. Delivery of DP has been evaluated using intermittent screening and treatment (IST) and IPTp, although the results for the IST approach have been disappointing. In studies conducted in Kenya and Malawi, compared to IPTp-SP, IST-DP was associated with a higher risk of maternal parasitaemia and PM [96, 97], and higher risk of abortions or stillbirths [97]. In contrast to IST-DP, IPTp-DP has shown promising results. In East Africa, IPTp-DP was shown to be associated with a lower risk of maternal parasitaemia and incidence of clinical malaria during pregnancy, and a lower risk of PM compared to IPTp-DP in HIV-uninfected pregnant women [36, 96]. However, despite the reduction in the burden of maternal malaria parasitaemia and PM, there was no significant improvement in birth outcomes observed in pregnant women who received IPTp-DP compared to those who received SP [37, 98].

1.7 Malaria burden and prevention in infants

Infants residing in areas of moderate-high malaria transmission intensity are one of the groups most at risk of malaria and its adverse effects. During the first 3 months of life, infants are partially protected from malaria by maternal IgG antibodies to *P. falciparum* acquired in utero [99, 100]. Infants older than 3 months of age are more likely to be admitted due to malaria and have a high risk of mortality if they develop severe malaria [2, 101]. As infants grow, the risk of malaria infection and clinical malaria increases from three months of age [102] as the levels of maternal IgG antibodies to *P. falciparum* begin to wane [103, 104]. Immunity to malaria after three months of life is acquired through repeated malaria infections.

To prevent and control malaria in infants, the main interventions include sleeping under insecticide treated nets, intermittent preventive treatment during infancy (IPTi) with SP [105], and seasonal malaria chemoprevention with SP in areas where malaria transmission is not perennial [106]. However, these interventions have limitations. The spread of vector resistance to pyrethroids, the insecticide used in bed nets [107], and the wide-spread antifolate resistance of malaria parasites especially in East and Central Africa [90] is threatening the effectiveness of these interventions. Additional ways of preventing and controlling malaria in infants are needed.

1.8 Effects of prevention of malaria in pregnancy on the risk of malaria in infants

Although PM has been reported to be associated with a higher risk of malaria during infancy [71-76] suggesting that prevention of malaria in pregnancy may have a protective effect against malaria during infancy, evidence of the impact of IPTp on the risk of malaria in infants is limited. A few studies have compared the risk of malaria in infants born to mothers receiving IPTp-SP

and alternative regimens have not shown a clear difference in effect. Compared to infants born to mothers receiving IPTp-SP, the incidence of malaria was similar among infants born to mothers randomised to IPTp-MQ [108] or IST with artemether lumefantrine (AL) [109]. However, these studies were limited by the failure of the alternative interventions (IPTp-MQ and IST-AL) to show a significant difference in the risk of PM compared to IPTp-SP [94, 110]. There is need to evaluate the impact of IPTp on the risk of malaria in infants in randomised trials using highly efficacious alternative drugs for IPTp.

In Uganda, a recent study compared the incidence of malaria in a birth cohort of infants born to mothers randomised to monthly IPTp-DP versus 2 monthly IPTp-DP versus 2 monthly IPTp-SP [111]. Surprisingly, in this birth cohort, monthly IPTp-DP was associated with a higher incidence of malaria in infants during the first year of life compared to 2 monthly IPTp-SP, but only in female infants. In male infants, monthly IPTp-DP was associated with a lower incidence of malaria compared to IPTp-SP, but the difference was not statistically significant. However, this study had several limitations. The sample size was small, infants received DP every three months for intermittent preventive treatment of malaria during infancy, and over the course of the study successful implementation of indoor residual spraying in the district substantially reduced malaria transmission [112]. Thus, there is need to evaluate the impact of IPTp-DP compared to SP on the risk of infants who are not taking drugs for malaria prevention in a high malaria transmission setting.

1.9 Thesis rationale

P. falciparum infection during pregnancy remains a major public health burden, which mainly affects pregnant women and children in malaria endemic areas. In pregnant women residing in areas of moderate to high malaria transmission intensity, *P. falciparum* infection is often asymptomatic but can lead to PM which is associated with maternal morbidity and adverse birth outcomes including preterm delivery, low birth weight and stillbirths [16, 17]. Furthermore, increasing evidence mainly from observational studies suggests that PM is associated with an increased risk of malaria in infancy [71-73, 75]. This suggests that prevention of malaria in pregnancy may lower the risk of malaria in infancy.

The WHO recommends IPTp-SP for pregnant women residing in areas of moderate-high malaria transmission intensity to prevent adverse effects of PM in both the mother and child [78]. However, the effectiveness of IPTp is threatened by wide-spread antifolate resistance [82]. Recent studies have shown DP to be a promising alternative to SP for IPTp. Compared to IPTp-SP, IPTp-DP was associated with a lower risk of malaria parasitaemia, and clinical malaria during

pregnancy and a lower risk of PM in HIV-uninfected pregnant women, but this did not significantly reduce the risk of adverse birth outcomes compared IPTp-SP [36, 96]. Because IPTp-DP reduces the risk of PM which has been reportedly associated with a higher risk of malaria infancy, it is hypothesised that IPTp with DP may have the additional benefit of reducing the risk of malaria in infants. However, evidence to support this hypothesis is currently limited.

This thesis further explores the association between PM and the incidence of malaria in infants; evaluates the impact of IPTp on the risk of malaria in infancy by comparing the incidence of malaria during the first year of life in infants born to HIV-uninfected mothers who were randomised to monthly IPTp with DP versus SP for prevention of malaria in pregnancy; and finally evaluates the impact of IPTp and PM on total cord blood *P. falciparum* IgG antibody levels.

1.10 Research questions

This thesis aimed to answer the following questions:

1) Is PM associated with a higher incidence of malaria during infancy?
2) Does preventing malaria in pregnancy with IPTp with DP, a highly efficacious antimalarial, protect against malaria during infancy more effectively than IPTp-SP?
3) What is the impact of PM and IPTp on total *P. falciparum*-specific IgG antibodies in cord blood?

1.11 Specific thesis objectives

1) To compare the incidence of malaria in infants during the first year of life among infants born to mothers with PM detected by histology and infants born to mothers without PM detected by histology.

2) To compare the incidence of malaria during the first year of life in infants born to mothers who were randomised to receive monthly IPTp-DP versus monthly IPTp-SP.

3) To evaluate the effect of PM and IPTp on cord blood levels of IgG antibodies to *P. falciparum* malaria antigens.

1.12 Study structure

This study was part of double-blind, randomised, controlled trial of monthly IPTp with DP versus SP for prevention of malaria in HIV-uninfected pregnant women and infants born to these women. The trial involved two phases: the pregnancy and infant phases. In the pregnancy phase, women were enrolled and followed up through delivery. The primary objective of the pregnancy

phase of the study was to compare the risk of a composite birth outcome (preterm delivery [<37 weeks of gestation], low birth weight [<2500g], or small for gestation age) among mothers who received IPTp-DP and mothers who received IPTp-SP. The findings of the pregnancy phase of the study have been published [37] and are not a subject of this thesis.

The infant phase, which is the focus of this thesis, involved follow-up of all live births to mothers who took part in the randomised trial to one year of age. The primary objective of the infant phase of the study was to compare the incidence of malaria during the first year of life among infants born to mothers randomised to monthly IPTp-DP with those born to mothers randomised to monthly IPTp-SP.

1.13 Thesis structure

Chapter 1 provides thesis introduction, thesis rationale, study questions, thesis objectives and a summary of the study structure. **Chapter 2** presents a systematic review of the literature on the impact of *P. falciparum* malaria and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants which was published in the Malaria Journal in September 2019 and an update of literature published since then**. Chapter 3** presents the study design and methodology including study procedures and statistical methods. In **Chapter 4**, a manuscript reporting the association between PM and the incidence of malaria in infants is presented. This manuscript was published in the Malaria Journal. **Chapter 5** presents a manuscript reporting the impact of IPTp-DP vs IPTp SP on the incidence of malaria in infants. This manuscript was published in the BMC Medicine journal. **Chapter 6** resents a manuscript on the effect of IPTp and PM on cord blood *P. falciparum* IgG antibody levels and the relationship between cord blood IgG antibody levels and the incidence of malaria during the first year of life. Lastly, **Chapter 7** discusses the research findings, their implications, and highlights areas for future research.

1.14 Publications from this thesis

1) Kakuru A, Staedke SG, Dorsey G, Rogerson S, Chandramohan D. Impact of *Plasmodium falciparum* malaria and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants: a systematic review. *Malar J*. 2019;18(1):304.
2) Kakuru A, Jagannathan P, Kajubi R, Ochieng T, Ochokoru H, Nakalembe M, Clark TD, Ruel T, Staedke SG, Chandramohan D, et al: Impact of intermittent preventive treatment of malaria in pregnancy with dihydroartemisinin-piperaquine versus sulfadoxine-pyrimethamine on the incidence of malaria in infancy: a randomized controlled trial. *BMC Med.* 2020, 18:207.

3) Kakuru A, Roh ME, Kajubi R, Ochieng T, Ategeka J, Ochokoru H, Nakalembe M, Clark TD, Ruel T, Staedke SG, Chandramohan D, Havlir DV, Kamya MR, Dorsey G, Jagannathan P. Infant sex modifies associations between placental malaria and risk of malaria in infancy. *Malar J.* 2020 Dec 3;19(1):449.
4) Kakuru A, Zehner N, Kajubi R, Staedke SG, Chandramohan D, Kamya MR, Dorsey G, Ssewanyana I, Jagannathan P. Association between placental malaria, intermittent preventive treatment of malaria in pregnancy and maternal-foetal transfer of antibodies to *P. falciparum.* In preparation

1.16 References

1. World Health Organization: **World malaria report 2019** [https://www.who.int/publications-detail/world-malaria-report-2019]. Accessed 21 Mar 2020.

2. Carneiro I, Roca-Feltrer A, Griffin JT, Smith L, Tanner M, Schellenberg JA, Greenwood B, Schellenberg D: **Age-patterns of malaria vary with severity, transmission intensity and seasonality in sub-Saharan Africa: a systematic review and pooled analysis.** *PLoS One* 2010, **5:**e8988.

3. Griffin JT, Hollingsworth TD, Reyburn H, Drakeley CJ, Riley EM, Ghani AC: **Gradual acquisition of immunity to severe malaria with increasing exposure.** *Proc Biol Sci* 2015, **282:**20142657.

4. Rodriguez-Barraquer I, Arinaitwe E, Jagannathan P, Kamya MR, Rosenthal PJ, Rek J, Dorsey G, Nankabirwa J, Staedke SG, Kilama M, et al: **Quantification of anti-parasite and anti-disease immunity to malaria as a function of age and exposure.** *Elife* 2018, **7**.

5. Rodriguez-Barraquer I, Arinaitwe E, Jagannathan P, Boyle MJ, Tappero J, Muhindo M, Kamya MR, Dorsey G, Drakeley C, Ssewanyana I, et al: **Quantifying heterogeneous malaria exposure and clinical protection in a cohort of Ugandan children.** *J Infect Dis* 2016, **214:**1072-1080.

6. Rahimi BA, Thakkinstian A, White NJ, Sirivichayakul C, Dondorp AM, Chokejindachai W: **Severe vivax malaria: a systematic review and meta-analysis of clinical studies since 1900.** *Malar J* 2014, **13:**481.

7. Naing C, Whittaker MA, Nyunt Wai V, Mak JW: **Is *Plasmodium vivax* malaria a severe malaria?: a systematic review and meta-analysis.** *PLoS Negl Trop Dis* 2014, **8:**e3071.

8. Poespoprodjo JR, Fobia W, Kenangalem E, Lampah DA, Warikar N, Seal A, McGready R, Sugiarto P, Tjitra E, Anstey NM, Price RN: **Adverse pregnancy outcomes in an area where multidrug-resistant *Plasmodium vivax* and *Plasmodium falciparum* infections are endemic.** *Clin Infect Dis* 2008, **46:**1374-1381.

9. Rijken MJ, McGready R, Boel ME, Poespoprodjo R, Singh N, Syafruddin D, Rogerson S, Nosten F: **Malaria in pregnancy in the Asia-Pacific region.** *The Lancet Infectious Diseases* 2012, **12:**75-88.

10. Saravu K, Rishikesh K, Kamath A, Shastry AB: **Severity in *Plasmodium vivax* malaria claiming global vigilance and exploration--a tertiary care centre-based cohort study.** *Malar J* 2014, **13:**304.

11. Bouyou-Akotet MK, Adegnika AA, Agnandji ST, Ngou-Milama E, Kombila M, Kremsner PG, Mavoungou E: **Cortisol and susceptibility to malaria during pregnancy.** *Microbes Infect* 2005, **7:**1217-1223.

12. Bouyou-Akotet MK, Issifou S, Meye JF, Kombila M, Ngou-Milama E, Luty AJ, Kremsner PG, Mavoungou E: **Depressed natural killer cell cytotoxicity against *Plasmodium falciparum*-infected erythrocytes during first pregnancies.** *Clin Infect Dis* 2004, **38:**342-347.

13. Lindsay S, Ansell J, Selman C, Cox V, Hamilton K, Walraven G: **Effect of pregnancy on exposure to malaria mosquitoes.** *The Lancet* 2000, **355:**1972.

14. Nosten F, Rogerson SJ, Beeson JG, McGready R, Mutabingwa TK, Brabin B: **Malaria in pregnancy and the endemicity spectrum: what can we learn?** *Trends in Parasitology* 2004, **20:**425-432.

15. Duffy PE: **Plasmodium in the placenta: parasites, parity, protection, prevention and possibly preeclampsia.** *Parasitology* 2007, **134:**1877-1881.

16. Moore KA, Simpson JA, Scoullar MJL, McGready R, Fowkes FJI: **Quantification of the association between malaria in pregnancy and stillbirth: a systematic review and meta-analysis.** *Lancet Glob Health* 2017, **5:**e1101-e1112.

17. Thompson JM, Eick SM, Dailey C, Dale AP, Mehta M, Nair A, Cordero JF, Welton M: **Relationship between pregnancy-associated malaria and adverse pregnancy outcomes: a systematic review and meta-analysis.** *J Trop Pediatr* 2019.

18. Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE: **Maternal antibodies block malaria.** *Nature* 1998, **395:**851-852.

19. Salanti A, Staalsoe T, Lavstsen T, Jensen AT, Sowa MP, Arnot DE, Hviid L, Theander TG: **Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering *Plasmodium falciparum* involved in pregnancy-associated malaria.** *Mol Microbiol* 2003, **49:**179-191.

20. Beeson JG, Brown GV, Molyneux ME, Mhango C, Dzinjalamala F, Rogerson SJ: ***Plasmodium falciparum*** **isolates from infected pregnant women and children are associated with distinct adhesive and antigenic properties.** *J Infect Dis* 1999, **180:**464-472.

21. Hviid L, Salanti A: **VAR2CSA and protective immunity against pregnancy-associated** ***Plasmodium falciparum*** **malaria.** *Parasitology* 2007, **134:**1871-1876.

22. Riley EM, Schneider G, Sambou I, Greenwood BM: **Suppression of cell-mediated immune responses to malaria antigens in pregnant Gambian women.** *Am J Trop Med Hyg* 1989, **40:**141-144.

23. Fievet N, Tami G, Maubert B, Moussa M, Shaw IK, Cot M, Holder AA, Chaouat G, Deloron P: **Cellular immune response to** ***Plasmodium falciparum*** **after pregnancy is related to previous placental infection and parity.** *Malar J* 2002, **1:**16.

24. Sherman IW, Eda S, Winograd E: **Cytoadherence and sequestration in** ***Plasmodium falciparum*****: defining the ties that bind.** *Microbes Infect* 2003, **5:**897-909.

25. Craig A, Scherf A: **Molecules on the surface of the** ***Plasmodium falciparum*** **infected erythrocyte and their role in malaria pathogenesis and immune evasion.** *Mol Biochem Parasitol* 2001, **115:**129-143.

26. Hviid L, Jensen AT: **PfEMP1 - a parasite protein family of key importance in** ***Plasmodium falciparum*** **malaria immunity and pathogenesis.** *Adv Parasitol* 2015, **88:**51-84.

27. David PH, Hommel M, Miller LH, Udeinya IJ, Oligino LD: **Parasite sequestration in** ***Plasmodium falciparum*** **malaria: spleen and antibody modulation of cytoadherence of infected erythrocytes.** *Proc Natl Acad Sci U S A* 1983, **80:**5075-5079.

28. Wahlgren M, Goel S, Akhouri RR: **Variant surface antigens of** ***Plasmodium falciparum*** **and their roles in severe malaria.** *Nat Rev Microbiol* 2017, **15:**479-491.

29. Muthusamy A, Achur RN, Bhavanandan VP, Fouda GG, Taylor DW, Gowda DC: ***Plasmodium falciparum*****-infected erythrocytes adhere both in the intervillous space and on the villous surface of human placenta by binding to the low-sulfated chondroitin sulfate proteoglycan receptor.** *Am J Pathol* 2004, **164:**2013-2025.

30. Walter PR, Garin Y, Blot P: **Placental pathologic changes in malaria. A histologic and ultrastructural study.** *Am J Pathol* 1982, **109:**330-342.

31. Watkinson M, Rushton DI: **Plasmodial pigmentation of placenta and outcome of pregnancy in West African mothers.** *Br Med J (Clin Res Ed)* 1983, **287:**251-254.

32. Fried M, Duffy PE: **Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta.** *Science* 1996, **272:**1502-1504.

33. Gowda DC, Ockenhouse CF: **Adherence of *Plasmodium falciparum*-infected erythrocytes to chondroitin 4-sulfate.** *Biosci Rep* 1999, **19:**261-271.

34. Brabin BJ, Romagosa C, Abdelgalil S, Menendez C, Verhoeff FH, McGready R, Fletcher KA, Owens S, D'Alessandro U, Nosten F, et al: **The sick placenta-the role of malaria.** *Placenta* 2004, **25:**359-378.

35. Kattenberg JH, Ochodo EA, Boer KR, Schallig HD, Mens PF, Leeflang MM: **Systematic review and meta-analysis: rapid diagnostic tests versus placental histology, microscopy and PCR for malaria in pregnant women.** *Malar J* 2011, **10:**321.

36. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, Opira B, Olwoch P, Ategeka J, Nayebare P, et al: **Dihydroartemisinin-piperaquine for the prevention of malaria in pregnancy.** *N Engl J Med* 2016, **374:**928-939.

37. Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, Ruel T, Opira B, Ochokoru H, Ategeka J, Nayebare P, et al: **Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in pregnancy: a double-blind, randomised, controlled, superiority trial.** *Lancet* 2019, **393:**1428-1439.

38. Mayor A, Moro L, Aguilar R, Bardaji A, Cistero P, Serra-Casas E, Sigauque B, Alonso PL, Ordi J, Menendez C: **How hidden can malaria be in pregnant women? Diagnosis by microscopy, placental histology, polymerase chain reaction and detection of histidine-rich protein 2 in plasma.** *Clin Infect Dis* 2012, **54:**1561-1568.

39. Rogerson SJ, Mkundika P, Kanjala MK: **Diagnosis of *Plasmodium falciparum* malaria at delivery: comparison of blood film preparation methods and of blood films with histology.** *J Clin Microbiol* 2003, **41:**1370-1374.

40. Bulmer JN, Rasheed FN, Francis N, Morrison L, Greenwood BM: **Placental malaria. I. Pathological classification.** *Histopathology* 1993, **22:**211-218.

41. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, Hirt R, Cardesa A, Alonso PL: **Placental pathology in malaria: a histological, immunohistochemical, and quantitative study.** *Hum Pathol* 2000, **31:**85-93.

42. Rogerson SJ, Pollina E, Getachew A, Tadesse E, Lema VM, Molyneux ME: **Placental monocyte infiltrates in response to *Plasmodium falciparum* malaria infection and their association with adverse pregnancy outcomes.** *Am J Trop Med Hyg* 2003, **68:**115-119.

43. Muehlenbachs A, Fried M, McGready R, Harrington Whitney E, Mutabingwa Theonest K, Nosten F, Duffy Patrick E: **A novel histological grading scheme for placental malaria applied in areas of high and low malaria transmission.** *The Journal of Infectious Diseases* 2010, **202:**1608-1616.

44. Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, Kahigwa E, Font F, Alonso PL: **The impact of placental malaria on gestational age and birth weight.** *J Infect Dis* 2000, **181:**1740-1745.

45. Umbers AJ, Aitken EH, Rogerson SJ: **Malaria in pregnancy: small babies, big problem.** *Trends Parasitol* 2011, **27:**168-175.

46. Omer SA, Idress HE, Adam I, Abdelrahim M, Noureldein AN, Abdelrazig AM, Elhassan MO, Sulaiman SM: **Placental malaria and its effect on pregnancy outcomes in Sudanese women from Blue Nile State.** *Malar J* 2017, **16:**374.

47. Tako EA, Zhou A, Lohoue J, Leke R, Taylor DW, Leke RF: **Risk factors for placental malaria and its effect on pregnancy outcome in Yaounde, Cameroon.** *Am J Trop Med Hyg* 2005, **72:**236-242.

48. Rahman MM, Abe SK, Rahman MS, Kanda M, Narita S, Bilano V, Ota E, Gilmour S, Shibuya K: **Maternal anemia and risk of adverse birth and health outcomes in low- and middle-income countries: systematic review and meta-analysis.** *Am J Clin Nutr* 2016, **103:**495-504.

49. Brabin BJ, Johnson PM: **Placental malaria and pre-eclampsia through the looking glass backwards?** *J Reprod Immunol* 2005, **65:**1-15.

50. Umbers AJ, Boeuf P, Clapham C, Stanisic DI, Baiwog F, Mueller I, Siba P, King CL, Beeson JG, Glazier J, Rogerson SJ: **Placental malaria-associated inflammation disturbs the insulin-like growth factor axis of fetal growth regulation.** *J Infect Dis* 2011, **203:**561-569.

51. Broen K, Brustoski K, Engelmann I, Luty AJF: **Placental *Plasmodium falciparum* infection: causes and consequences of in utero sensitization to parasite antigens.** *Mol Biochem Parasitol* 2007, **151:**1-8.

52. Dauby N, Goetghebuer T, Kollmann TR, Levy J, Marchant A: **Uninfected but not unaffected: chronic maternal infections during pregnancy, fetal immunity, and susceptibility to postnatal infections.** *Lancet Infect Dis* 2012, **12:**330-340.

53. Malhotra I, Dent A, Mungai P, Wamachi A, Ouma JH, Narum DL, Muchiri E, Tisch DJ, King CL: **Can prenatal malaria exposure produce an immune tolerant phenotype? A prospective birth cohort study in Kenya.** *PLoS Med* 2009, **6:**e1000116.

54. Malhotra I, Mungai P, Muchiri E, Ouma J, Sharma S, Kazura JW, King CL: **Distinct Th1- and Th2-Type prenatal cytokine responses to *Plasmodium falciparum* erythrocyte invasion ligands.** *Infect Immun* 2005, **73:**3462-3470.

55. McKittrick ND, Vu DM, Malhotra I, King CH, Mutuku F, LaBeaud AD: **Parasitic Infections in pregnancy decrease placental transfer of antipneumococcus antibodies.** *Clin Vaccine Immunol* 2017, **24**.

56. Bisseye C, van der Sande M, Morgan WD, Holder AA, Pinder M, Ismaili J: ***Plasmodium falciparum* infection of the placenta impacts on the T helper type 1 (Th1)/Th2 balance of neonatal T cells through CD4(+)CD25(+) forkhead box P3(+) regulatory T cells and interleukin-10.** *Clin Exp Immunol* 2009, **158:**287-293.

57. Fievet N, Varani S, Ibitokou S, Briand V, Louis S, Perrin RX, Massougbogji A, Hosmalin A, Troye-Blomberg M, Deloron P: ***Plasmodium falciparum* exposure in utero, maternal age and parity influence the innate activation of foetal antigen presenting cells.** *Malar J* 2009, **8:**251.

58. Gbedande K, Varani S, Ibitokou S, Houngbegnon P, Borgella S, Nouatin O, Ezinmegnon S, Adeothy AL, Cottrell G, Massougbodji A, et al: **Malaria modifies neonatal and early-life toll-like receptor cytokine responses.** *Infect Immun* 2013, **81:**2686-2696.

59. Achidi EA, Anchang JK, Minang JT, Ahmadou MJ, Troye-Blomberg M: **Studies on *Plasmodium falciparum* isotypic antibodies and numbers of IL-4 and IFN- gamma secreting cells in paired maternal cord blood from South West Cameroon.** *Int J Infect Dis* 2005, **9:**159-169.

60. Ismaili J, Van Der Sande M, Holland MJ, Sambou I, Keita S, Allsopp C, Ota MO, McAdam KPWJ, Pinder M: ***Plasmodium falciparum*** **infection of the placenta affects newborn immune responses.** *Clin Exp Immunol* 2003, **133:**414-421.

61. Brustoski K, Moller U, Kramer M, Hartgers FC, Kremsner PG, Krzych U, Luty AJ: **Reduced cord blood immune effector-cell responsiveness mediated by CD4+ cells induced in utero as a consequence of placental *Plasmodium falciparum* infection.** *J Infect Dis* 2006, **193:**146-154.

62. Engelmann I, Santamaria A, Kremsner PG, Luty AJ: **Activation status of cord blood gamma delta T cells reflects in utero exposure to *Plasmodium falciparum* antigen.** *J Infect Dis* 2005, **191:**1612-1622.

63. Okoko BJ, Wesumperuma LH, Ota MO, Pinder M, Banya W, Gomez SF, McAdam KP, Hart AC: **The influence of placental malaria infection and maternal hypergammaglobulinemia on transplacental transfer of antibodies and IgG subclasses in a rural West African population.** *J Infect Dis* 2001, **184:**627-632.

64. Brair ME, Brabin BJ, Milligan P, Maxwell S, Hart CA: **Reduced transfer of tetanus antibodies with placental malaria.** *Lancet* 1994, **343:**208-209.

65. Cumberland P, Shulman CE, Maple PA, Bulmer JN, Dorman EK, Kawuondo K, Marsh K, Cutts FT: **Maternal HIV infection and placental malaria reduce transplacental antibody transfer and tetanus antibody levels in newborns in Kenya.** *J Infect Dis* 2007, **196:**550-557.

66. de Moraes-Pinto MI, Verhoeff F, Chimsuku L, Milligan PJ, Wesumperuma L, Broadhead RL, Brabin BJ, Johnson PM, Hart CA: **Placental antibody transfer: influence of maternal HIV infection and placental malaria.** *Arch Dis Child Fetal Neonatal Ed* 1998, **79:**F202-205.

67. Dechavanne C, Cottrell G, Garcia A, Migot-Nabias F: **Placental Malaria: decreased transfer of maternal antibodies directed to *Plasmodium falciparum* and impact on the incidence of febrile infections in infants.** *PLoS ONE [Electronic Resource]* 2015, **10:**e0145464.

68. Moro L, Bardaji A, Nhampossa T, Mandomando I, Serra-Casas E, Sigauque B, Cistero P, Chauhan VS, Chitnis CE, Ordi J, et al: **Malaria and HIV infection in Mozambican pregnant women are associated with reduced transfer of antimalarial antibodies to their newborns.** *J Infect Dis* 2015, **211:**1004-1014.

69. Rachas A, Le Port A, Cottrell G, Guerra J, Choudat I, Bouscaillou J, Massougbodji A, Garcia A: **Placental malaria is associated with increased risk of nonmalaria infection during the first 18 months of life in a Beninese population.** *Clin Infect Dis* 2012, **55:**672-678.

70. Bonner PC, Zhou Z, Mirel LB, Ayisi JG, Shi YP, van Eijk AM, Otieno JA, Nahlen BL, Steketee RW, Udhayakumar V: **Placental malaria diminishes development of antibody responses to *Plasmodium falciparum* epitopes in infants residing in an area of western Kenya where *P. falciparum* is endemic.** *Clin Diagn Lab Immunol* 2005, **12:**375-379.

71. Boudova S, Divala T, Mungwira R, Mawindo P, Tomoka T, Laufer MK: **Placental but not peripheral *Plasmodium falciparum* infection during pregnancy is associated with increased risk of malaria in infancy.** *J Infect Dis* 2017, **216:**732-735.

72. De Beaudrap P, Turyakira E, Nabasumba C, Tumwebaze B, Piola P, Boum Ii Y, McGready R: **Timing of malaria in pregnancy and impact on infant growth and morbidity: a cohort study in Uganda.** *Malar J* 2016, **15:**92.

73. Le Port A, Watier L, Cottrell G, Ouedraogo S, Dechavanne C, Pierrat C, Rachas A, Bouscaillou J, Bouraima A, Massougbodji A, et al: **Infections in infants during the first 12 months of life: role of placental malaria and environmental factors.** *PLoS ONE [Electronic Resource]* 2011, **6:**e27516.

74. Schwarz NG, Adegnika AA, Breitling LP, Gabor J, Agnandji ST, Newman RD, Lell B, Issifou S, Yazdanbakhsh M, Luty AJ, et al: **Placental malaria increases malaria risk in the first 30 months of life.** *Clin Infect Dis* 2008, **47:**1017-1025.

75. Sylvester B, Gasarasi DB, Aboud S, Tarimo D, Massawe S, Mpembeni R, Swedberg G: **Prenatal exposure to *Plasmodium falciparum* increases frequency and shortens time from birth to first clinical malaria episodes during the first two years of life: prospective birth cohort study.** *Malar J* 2016, **15:**379.

76. Tassi Yunga S, Fouda GG, Sama G, Ngu JB, Leke RGF, Taylor DW: **Increased susceptibility to *Plasmodium falciparum* in Infants is associated with low, not high, placental malaria parasitemia.** *Sci Rep* 2018, **8:**169.

77. Cairns M, Gosling R, Chandramohan D: **Placental malaria increases malaria risk in the first 30 months of life: not causal.** *Clin Infect Dis* 2009, **48:**497-498; author reply 498-499.

78. World Health Organization: **WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP).** [http://www.who.int/malaria/publications/atoz/iptp-sp-updated-policy-brief-24jan2014.pdf]

79. Schultz LJ, Steketee RW, Macheso A, Kazembe P, Chitsulo L, Wirima JJ: **The efficacy of antimalarial regimens containing sulfadoxine-pyrimethamine and/or chloroquine in preventing peripheral and placental *Plasmodium falciparum* infection among pregnant women in Malawi.** *Am J Trop Med Hyg* 1994, **51:**515-522.

80. Shulman CE, Dorman EK, Cutts F, Kawuondo K, Bulmer JN, Peshu N, Marsh K: **Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: a randomised placebo-controlled trial.** *Lancet* 1999, **353:**632-636.

81. Verhoeff FH, Brabin BJ, Chimsuku L, Kazembe P, Russell WB, Broadhead RL: **An evaluation of the effects of intermittent sulfadoxine-pyrimethamine treatment in pregnancy on parasite clearance and risk of low birthweight in rural Malawi.** *Ann Trop Med Parasitol* 1998, **92:**141-150.

82. Naidoo I, Roper C: **Drug resistance maps to guide intermittent preventive treatment of malaria in African infants.** *Parasitology* 2011, **138:**1469-1479.

83. Kyabayinze D, Cattamanchi A, Kamya MR, Rosenthal PJ, Dorsey G: **Validation of a simplified method for using molecular markers to predict sulfadoxine-pyrimethamine treatment failure in African children with *falciparum* malaria.** *Am J Trop Med Hyg* 2003, **69:**247-252.

84. Desai M, Gutman J, Taylor SM, Wiegand RE, Khairallah C, Kayentao K, Ouma P, Coulibaly SO, Kalilani L, Mace KE, et al: **Impact of sulfadoxine-pyrimethamine resistance on effectiveness of intermittent preventive therapy for malaria in pregnancy at clearing infections and preventing low birth weight.** *Clin Infect Dis* 2016, **62:**323-333.

85. Gutman J, Mwandama D, Wiegand RE, Abdallah J, Iriemenam NC, Shi YP, Mathanga DP, Skarbinski J: **In vivo efficacy of sulphadoxine-pyrimethamine for the treatment of asymptomatic parasitaemia in pregnant women in Machinga District, Malawi.** *Malar J* 2015, **14:**197.

86. Moussiliou A, De Tove YS-S, Doritchamou J, Luty AJF, Massougbodji A, Alifrangis M, Deloron P, Ndam NT: **High rates of parasite recrudescence following intermittent preventive**

treatment with sulphadoxine-pyrimethamine during pregnancy in Benin. *Malar J* 2013, **12:**195-195.

87. Okell LC, Griffin JT, Roper C: **Mapping sulphadoxine-pyrimethamine-resistant *Plasmodium falciparum* malaria in infected humans and in parasite populations in Africa.** *Sci Rep* 2017, **7**.

88. Kayentao K, Garner P, van Eijk AM, Naidoo I, Roper C, Mulokozi A, MacArthur JR, Luntamo M, Ashorn P, Doumbo OK, ter Kuile FO: **Intermittent preventive therapy for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in Africa: systematic review and meta-analysis.** *JAMA* 2013, **309:**594-604.

89. Amimo F, Lambert B, Magit A, Sacarlal J, Hashizume M, Shibuya K: ***Plasmodium falciparum* resistance to sulfadoxine-pyrimethamine in Africa: a systematic analysis of national trends.** *BMJ Glob Health* 2020, **5**.

90. van Eijk AM, Larsen DA, Kayentao K, Koshy G, Slaughter DEC, Roper C, Okell LC, Desai M, Gutman J, Khairallah C, et al: **Effect of *Plasmodium falciparum* sulfadoxine-pyrimethamine resistance on the effectiveness of intermittent preventive therapy for malaria in pregnancy in Africa: a systematic review and meta-analysis.** *The Lancet Infectious Diseases* 2019.

91. Gutman J, Kalilani L, Taylor S, Zhou Z, Wiegand RE, Thwai KL, Mwandama D, Khairallah C, Madanitsa M, Chaluluka E, et al: **The A581G mutation in the gene encoding *Plasmodium falciparum* dihydropteroate synthetase reduces the effectiveness of sulfadoxine-pyrimethamine preventive therapy in Malawian pregnant women.** *J Infect Dis* 2015, **211:**1997-2005.

92. Chico RM, Cano J, Ariti C, Collier TJ, Chandramohan D, Roper C, Greenwood B: **Influence of malaria transmission intensity and the 581G mutation on the efficacy of intermittent preventive treatment in pregnancy: systematic review and meta-analysis.** *Trop Med Int Health* 2015, **20:**1621-1633.

93. Clerk CA, Bruce J, Affipunguh PK, Mensah N, Hodgson A, Greenwood B, Chandramohan D: **A randomized, controlled trial of intermittent preventive treatment with sulfadoxine-pyrimethamine, amodiaquine, or the combination in pregnant women in Ghana.** *J Infect Dis* 2008, **198:**1202-1211.

94. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, Kakolwa MA, Abdulla S, Accrombessi M, Aponte JJ, Akerey-Diop D, Basra A, Briand V, et al: **Intermittent preventive treatment of**

malaria in pregnancy with mefloquine in HIV-negative women: a multicentre randomized controlled trial. *PLoS Med* 2014, **11:**e1001733.

95. Kimani J, Phiri K, Kamiza S, Duparc S, Ayoub A, Rojo R, Robbins J, Orrico R, Vandenbroucke P: **Efficacy and safety of azithromycin-chloroquine versus sulfadoxine-pyrimethamine for intermittent preventive treatment of *Plasmodium falciparum* malaria infection in pregnant women in Africa: an open-label, randomized trial.** *PLoS One* 2016, **11:**e0157045.

96. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, Ouma P, Were V, Laserson K, Katana A, et al: **Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial.** *Lancet* 2015, **386:**2507-2519.

97. Madanitsa M, Kalilani L, Mwapasa V, van Eijk AM, Khairallah C, Ali D, Pace C, Smedley J, Thwai KL, Levitt B, et al: **Scheduled intermittent screening with rapid diagnostic tests and treatment with dihydroartemisinin-piperaquine versus intermittent preventive therapy with sulfadoxine-pyrimethamine for malaria in pregnancy in Malawi: an open-label randomized controlled trial.** *PLoS Med* 2016, **13:**e1002124.

98. Roh ME, Kuile FOT, Rerolle F, Glymour MM, Shiboski S, Gosling R, Gutman J, Kakuru A, Desai M, Kajubi R, et al: **Overall, anti-malarial, and non-malarial effect of intermittent preventive treatment during pregnancy with sulfadoxine-pyrimethamine on birthweight: a mediation analysis.** *Lancet Glob Health* 2020, **8:**e942-e953.

99. Dobbs KR, Dent AE: ***Plasmodium* malaria and antimalarial antibodies in the first year of life.** *Parasitology* 2016, **143:**129-138.

100. Riley EM, Wagner GE, Akanmori BD, Koram KA: **Do maternally acquired antibodies protect infants from malaria infection?** *Parasite Immunol* 2001, **23:**51-59.

101. Reyburn H, Mbatia R, Drakeley C, Bruce J, Carneiro I, Olomi R, Cox J, Nkya WM, Lemnge M, Greenwood BM, Riley EM: **Association of transmission intensity and age with clinical manifestations and case fatality of severe *Plasmodium falciparum* malaria.** *JAMA* 2005, **293:**1461-1470.

102. Bloland PB, Boriga DA, Ruebush TK, McCormick JB, Roberts JM, Oloo AJ, Hawley W, Lal A, Nahlen B, Campbell CC: **Longitudinal cohort study of the epidemiology of malaria**

infections in an area of intense malaria transmission II. Descriptive epidemiology of malaria infection and disease among children. *Am J Trop Med Hyg* 1999, **60:**641-648.

103. Reynaldi A, Dent AE, Schlub TE, Ogolla S, Rochford R, Davenport MP: **Interaction between maternally derived antibodies and heterogeneity in exposure combined to determine time-to-first *Plasmodium falciparum* infection in Kenyan infants.** *Malar J* 2019, **18:**19.

104. Sehgal VM, Siddjiqui WA, Alpers MP: **A seroepidemiological study to evaluate the role of passive maternal immunity to malaria in infants.** *Trans R Soc Trop Med Hyg* 1989, **83 Suppl:**105-106.

105. Esu EB, Oringanje C, Meremikwu MM: **Intermittent preventive treatment for malaria in infants.** *Cochrane Database Syst Rev* 2019, **12:**Cd011525.

106. World Health Organization: **Malaria prevention works: let's close the gap** [https://www.who.int/malaria/publications/atoz/malaria-prevention-works/en/]. Accessed 30 Jan 2021.

107. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V: **Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control?** *Trends Parasitol* 2011, **27:**91-98.

108. Ruperez M, Gonzalez R, Mombo-Ngoma G, Kabanywanyi AM, Sevene E, Ouedraogo S, Kakolwa MA, Vala A, Accrombessi M, Briand V, et al: **Mortality, morbidity, and developmental outcomes in infants born to women who received either mefloquine or sulfadoxine-pyrimethamine as intermittent preventive treatment of malaria in pregnancy: a cohort study.** *PLoS Medicine / Public Library of Science* 2016, **13:**e1001964.

109. Awine T, Belko MM, Oduro AR, Oyakhirome S, Tagbor H, Chandramohan D, Milligan P, Cairns M, Greenwood B, Williams JE: **The risk of malaria in Ghanaian infants born to women managed in pregnancy with intermittent screening and treatment for malaria or intermittent preventive treatment with sulfadoxine/pyrimethamine.** *Malar J* 2016, **15:**46.

110. Tagbor H, Cairns M, Bojang K, Coulibaly SO, Kayentao K, Williams J, Abubakar I, Akor F, Mohammed K, Bationo R, et al: **A non-inferiority, individually randomized trial of intermittent screening and treatment versus intermittent preventive treatment in the control of malaria in pregnancy.** *PLoS One* 2015, **10:**e0132247.

111. Jagannathan P, Kakuru A, Okiring J, Muhindo MK, Natureeba P, Nakalembe M, Opira B, Olwoch P, Nankya F, Ssewanyana I, et al: **Dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria during pregnancy and risk of malaria in early childhood: a randomized controlled trial.** *PLoS Med* 2018, **15:**e1002606.

112. Oguttu DW, Matovu JKB, Okumu DC, Ario AR, Okullo AE, Opigo J, Nankabirwa V: **Rapid reduction of malaria following introduction of vector control interventions in Tororo District, Uganda: a descriptive study.** *Malar J* 2017, **16:**227.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

This chapter presents a systematic review of the literature on the impact of *P. falciparum* malaria and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants which was published in Malaria Journal in September 2019. At the end of the chapter, an update of the recent published literature on the same subject and a conclusion are presented.

2.2 Systematic review paper

Below is the research paper cover sheet for the published systematic review, followed by the systematic review manuscript.

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1602857	**Title**	Dr
First Name(s)	Abel		
Surname/Family Name	Kakuru		
Thesis Title	Impact of malaria in pregnancy and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants		
Primary Supervisor	Sarah G. Staedke		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Malaria Journal		
When was the work published?	September 2019		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	Not applicable		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	

Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the systematic review, wrote the protocol, carried out the literature search, collected the data, conducted the data synthesis and wrote the first draft of the muscript

SECTION E

Student Signature	[redacted]
Date	21/09/2020

Supervisor Signature	[redacted]
Date	21 Sept 2020

Kakuru et al. Malar J (2019) 18:304
https://doi.org/10.1186/s12936-019-2943-3

Malaria Journal

RESEARCH Open Access

Impact of *Plasmodium falciparum* malaria and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants: a systematic review

Abel Kakuru[1,2*], Sarah G. Staedke[2], Grant Dorsey[3], Stephen Rogerson[4] and Daniel Chandramohan[2]

Abstract

Background: Studies of the association between malaria in pregnancy (MiP) and malaria during infancy have provided mixed results. A systematic review was conducted to evaluate available evidence on the impact of *Plasmodium falciparum* malaria infection during pregnancy, and intermittent preventive treatment of malaria during pregnancy (IPTp), on the risk of clinical malaria or parasitaemia during infancy.

Methods: MEDLINE, EMBASE, Global Health, and Malaria in Pregnancy Library databases were searched from inception to 22 May 2018 for articles published in English that reported on associations between MiP and malaria risk in infancy. Search terms included malaria, *Plasmodium falciparum*, pregnancy, placenta, maternal, prenatal, foetal, newborn, infant, child or offspring or preschool. Randomised controlled trials and prospective cohort studies, which followed infants for at least 6 months, were included if any of the following outcomes were reported: incidence of clinical malaria, prevalence of parasitaemia, and time to first episode of parasitaemia or clinical malaria. Substantial heterogeneity between studies precluded meta-analysis. Thus, a narrative synthesis of included studies was conducted.

Results: The search yielded 14 published studies, 10 prospective cohort studies and four randomised trials; all were conducted in sub-Saharan Africa. Infants born to mothers with parasitaemia during pregnancy were at higher risk of malaria in three of four studies that assessed this association. Placental malaria detected by microscopy or histology was associated with a higher risk of malaria during infancy in nine of 12 studies, but only one study adjusted for malaria transmission intensity. No statistically significant associations between the use of IPTp or different IPTp regimens and the risk of malaria during infancy were identified.

Conclusion: Evidence of an association between MiP and IPTp and risk of malaria in infancy is limited and of variable quality. Most studies did not adequately adjust for malaria transmission intensity shared by mothers and their infants. Further research is needed to confirm or exclude an association between MiP and malaria in infancy. Randomised trials evaluating highly effective interventions aimed at preventing MiP, such as IPTp with dihydroartemisinin-piperaquine, may help to establish a causal association between MiP and malaria in infancy.

Keywords: Malaria, pregnancy, infants, intermittent preventive treatment

---

*Correspondence: abelkakuru@gmail.com

2 London School of Hygiene and Tropical Medicine, Keppel Street,

London WC1E 7HT, UK

Full list of author information is available at the end of the article

Background

In sub-Saharan Africa, an estimated 30 million pregnant women are at risk of *Plasmodium falciparum* infection every year [1]. In areas of moderate to high malaria transmission intensity, like most parts of sub-Saharan Africa, *P. falciparum* infection in pregnant women is usually asymptomatic because adults are usually partially immune to malaria infection. However, *P. falciparum* infection during pregnancy can lead to placental malaria (PM). At delivery, 25% of pregnant women in sub-Saharan Africa were estimated to have PM detected by microscopy in 2007 [2]. Infection with *P. falciparum* during pregnancy has been associated with maternal morbidity such as maternal anaemia [3] and adverse birth outcomes including abortions, stillbirths, preterm delivery, and low birth weight [4-7].

The effects of *P. falciparum* infection during pregnancy on the infant may extend beyond the neonatal period [8]. Studies have shown that in utero foetal exposure to malaria antigens may negatively affect development of immunity to infectious diseases, including malaria in the newborn [9-11]. Foetal exposure to *P. falciparum* antigens has been shown to induce malaria specific immune responses that are biased towards tolerance to malaria antigens [12-14], while treatment of malaria in pregnancy (MiP) was shown to be associated with pro-inflammatory responses toward malaria specific antigens [15], suggesting that infants exposed to malaria in utero may have a higher risk of malaria during early childhood, and treatment of MiP may improve antimalarial immunity in infants. However, studies evaluating the association between MiP and malaria in infancy have shown mixed results. Some studies have reported an increased risk of clinical malaria or parasitaemia in infants born to mothers with placental malaria (PM) [16-18], while others have reported no difference in the risk of malaria in infants born to mothers with and without PM at delivery [19, 20]. One study has suggested that infants born to primigravid mothers with PM have a lower risk of malaria [21].

Intermittent preventive treatment of MiP (IPTp) with sulfadoxine-pyrimethamine (SP), remains one of the main interventions recommended by the World Health Organization in areas of moderate to high malaria transmission intensity mainly to improve birth outcomes [22], despite widespread resistance of malaria parasites to antifolate drugs [23]. Although IPTp-SP still improves birth outcomes in settings with antifolate resistance [24], its impact on PM and maternal parasitaemia remains minimal [25, 26]. This continues to expose the foetus to malaria antigens which may negatively affect the health of the infant even after delivery [27]. Intermittent preventive treatment has been shown to be associated with improved infant outcomes beyond delivery, such as perinatal mortality [28], but the impact of IPTp on the risk of malaria during infancy is not well known. With available promising alternative drugs for IPTp

such as dihydroartemisinin piperaquine (DP), which markedly reduces both the risk of malaria parasitaemia and incidence of clinical malaria during pregnancy, and reduces the prevalence of PM at delivery but does not clearly improve birth outcomes compared to IPTp-SP [25, 26, 29], possible additional benefits of IPTp to the newborn including reducing the risk of malaria in infancy may have IPTp policy implications. Understanding the impact of IPTp on the risk of malaria in infants is important in order to maximise the benefits of malaria prevention in pregnancy.

To better understand the effect of maternal parasitaemia, PM, and IPTp on the risk of malaria in infants, which may have potential to guide policy on the choice of alternative drugs for IPTp, a systematic review was conducted to examine and summarize published studies evaluating the impact of *P. falciparum* parasitaemia in pregnancy and PM, and the effect of IPTp, on the risk of clinical malaria or parasitaemia in infants.

Methods

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [30]. The protocol for this systematic review was developed and registered with PROSPERO register (Registration number: CRD42018088869) prior to conducting the review.

Selection criteria

Original research studies were included if they were published in English and evaluated associations between *P. falciparum* infection during pregnancy or IPTp and the risk of parasitaemia or incidence of malaria in infants born to HIV-uninfected pregnant women. Only randomised controlled trials (RCTs) and prospective cohort studies in which infants were followed up for at least 6 months were included. Studies involving only HIV-exposed infants, animal studies, and studies of non-falciparum malaria were excluded.

Information sources and search strategy

MEDLINE, EMBASE, Global Health, and Malaria in Pregnancy (MiP) Library [31] databases were searched from inception to 22 May 2018. All review authors participated in developing the search strategy and AK conducted the search. MEDLINE, EMBASE and Global Health databases were searched via the Ovid interface using Medical Subject Headings (MeSH) of key search terms. The MiP library [31] was searched using key search terms, including malaria, *Plasmodium falciparum*, falciparum malaria, pregnancy, placenta, maternal, foetal, prenatal, utero, new-

born, infant, child, offspring, and preschool. The search was limited to journal articles reporting human studies and published in the English language. Additional studies were identified by scrutinising reference lists of studies that met the inclusion criteria and relevant review articles. A bibliography of included studies was shared with other experts in the field of MiP to assess whether all the relevant articles had been retrieved.

Study selection

Lists of titles and abstracts of retrieved articles were exported to Endnote and duplicates were removed. Retrieved titles and abstracts were assessed for eligibility at the level of titles and abstracts by the corresponding author. Full articles and abstracts of potentially relevant articles and those where there was uncertainty about whether to include or exclude the article were retrieved and assessed for eligibility by two independent reviewers (AK and DC). Where there was disagreement, it was resolved by discussing with the rest of the authors.

Data extraction process

Data were extracted by one reviewer (AK) and verified by a second reviewer (DC). A data extraction matrix in an Excel spreadsheet was developed and piloted prior to data extraction. The Excel spreadsheet included the following variables: author and year of publication, country where the study was done, study period (dates of fieldwork), transmission intensity as measured by entomological inoculation rate, study design (considered RCT if IPTp was randomised, otherwise defined as an observational study), length of follow-up, follow-up schedule, study objectives, study population, sample size, long-lasting insecticide treated net (ITN) coverage among study participants, whether IPTp was given, type and frequency of IPTp regimen, whether maternal peripheral malaria parasitaemia was measured, timing of measurement of maternal peripheral malaria parasitaemia and how it was measured, PM detection, PM case definition, when and how clinical malaria or parasitaemia were detected in the infant, study outcomes, proportion of infants born to mothers with peripheral malaria parasitaemia or PM, adjustment for potential confounding factors, losses to follow-up, proportion of infants with outcomes of interest, results including effect sizes with confidence intervals and p-values, and study strengths and limitations. Disagreements between the two reviewers were resolved by discussion. Unresolved disagreements were settled by a third reviewer (either SS or GD or SR). Corresponding authors of included studies were contacted by email for any missing or unclear information. Corresponding authors who did not respond to the first email contact were contacted two more times and if they did not respond at the third contact, they were not contacted any further.

Assessment of risk of bias

The risk of bias in individual studies was assessed by two reviewers using the Newcastle-Ottawa quality assessment scale for cohort studies [32], and the Cochrane Collaboration tool for randomised controlled trials [33]. Details of how the risk of bias was assessed in cohort studies were published in the systematic review protocol (PROSPERO Registration number: CRD42018088869). Studies were rated on the following categories: selection, comparability, and outcome. For the selection category, studies were rated on the following items: representativeness of the exposed group (whether the exposed cohort was truly representative of the average from the community), selection of the non-exposed group, measurement of the exposure (if the exposed and the unexposed were from the same community), and demonstration that the outcome was not present at the start of follow-up. For the comparability category, studies were assessed based on whether the study adjusted for malaria transmission or controlled for malaria prevention during pregnancy using IPTp or ITNs. In the outcome category, studies were rated on the following items: ascertainment of outcome, whether follow-up was long enough for outcomes to occur (follow-up of six months was considered adequate), and completeness of follow-up (proportion of infants lost to follow-up, and whether characteristics of those lost to follow-up were reported). Each item in the selection and outcome categories was awarded a maximum of one point. The comparability category was awarded a maximum of two points. Studies were awarded a maximum of nine points. Studies that had a score of 9 were rated as having medium risk of bias while those with a score of ≤8 were rated as high risk.

Data synthesis

A systematic narrative synthesis of the included studies was conducted. A meta-analysis was not conducted because studies had substantial variation in the definition of malaria exposure during pregnancy, type of IPTp given, length of follow-up, and determination of primary outcome. Results were summarised using tables and in text. Tables with summary descriptions of study design, MiP exposure measurement, follow-up time, outcome measurement and results were generated. Studies were grouped in clusters according to the type of exposure (maternal peripheral malaria during pregnancy, or PM or IPTp), and outcome of interest (incidence of malaria in infants, prevalence of malaria parasitaemia, time to first malaria parasitaemia or first episode of malaria).

Results

Study selection

Overall, 2084 titles and abstracts were identified and retrieved from searches of electronic databases. An additional 2 records were identified from searching lists and contacting experts in the field (figure 2.1). Of the 2086 records, 461 duplicates were removed, and 1625 records were screened. Of these, 1582 records were excluded after review of the title and abstract, and 29 were excluded for various reasons after reviewing full text articles. Only 14 articles were deemed to be eligible for inclusion in the systematic review.

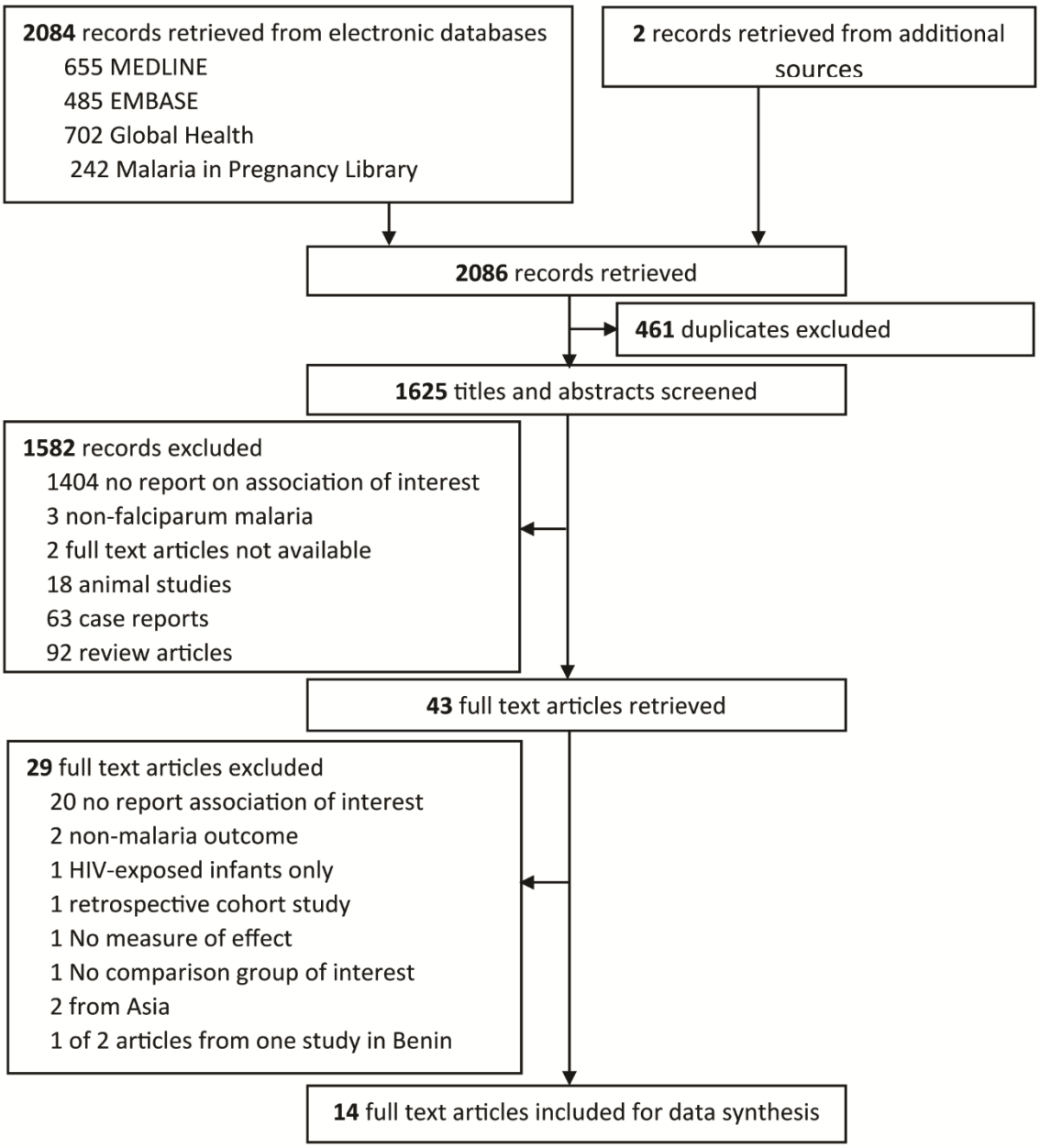

Figure 2.1 Study Selection results

Characteristics of included studies

All 14 studies were conducted in sub-Saharan Africa (Table 2.1); 10 prospective cohort studies and four RCTs. Of the 10 cohort studies, IPTp-SP was given in seven studies, and IPTp was not given in two [17, 34] because they were conducted before implementation of the WHO recommendation on IPTp. Malaria transmission data in form of entomological inoculation rate (number of infectious mosquito bites per person per year) was reported in half of the included studies; 20.5 [35], 35 [18], 38 [16], 50 [17], 257 [34], and 400 [19, 21]. The duration of follow-up

of infants ranged from one year to five years. In 10 studies, infants were followed up from birth to one year of age [16, 18-21, 34-38]. Maternal malaria exposure was determined by 1) microscopy (N=5) [18, 36, 37, 39] or DNA PCR of maternal blood [40], 2) microscopy of placental blood only (N=6) [17, 18, 21, 34, 35, 37], or 3) histology of placental tissue (N=5) [16, 19, 36, 40, 41]. Malaria infection during infancy was measured as malaria parasitaemia (prevalence of parasitaemia, N=7; time to first parasitaemia, N=4), and clinical malaria (incidence of clinical malaria, N=5; time to first clinical malaria, N=2). Malaria parasitaemia was assessed during weekly [35], fortnightly [21], or monthly [18, 37] routine visits. Clinical malaria was assessed by active surveillance in three studies [21, 35, 36] and by passive surveillance in 11 of the studies [16-20, 34, 37-41]. ITN use in infants was not reported in half of the studies. In five studies, ITN use was reported as 100% [20, 41], 51% [21], 73% [19], and 95% [18]. In one study, 66% of infants were from families that owned at least an ITN at enrolment [35] while in another study, all infants did not have ITNs [34]. The median number of infants born to mothers with maternal parasitaemia was 224 (range 28-236). The median number of infants born to mothers with PM detected by microscopy or histology was 59 (range 15-445). Out of 11 studies which assessed association between PM and the risk of malaria in infants, there were three studies with ≥100 infants born to mothers with PM.

Table 2.1 Characteristics of included studies

Author, year of publication. Country (reference)	N	Study design	IPTp regimens	Follow-up duration	Measures of malaria in pregnancy			Measures of malaria during infancy	
					Maternal blood[a]	Placental blood[b]	Placental histology[c]	Parasitaemia	Clinical malaria
Tassi Yunga, 2018. Cameroon [34]	80	cohort	None	1 year		✓		✓	
Boudova, 2017. Malawi [40]	473	RCT	SP vs CQ vs CQ prophylaxis	2 years	✓	✓	✓	✓	✓
Ruperez, 2016. Benin, Gabon, Tanzania, Mozambique [38]	4247	RCT	SP vs MQ	1 year					✓
Sylvester, 2016. Tanzania [41]	206	cohort	Not reported	2 years			✓	✓	
De Beaudrap, 2016. Uganda [37]	832	cohort	SP	1 year	✓	✓		✓	
Awine, 2016. Ghana [19]	988	RCT	SP vs ISTp-AL	1 year			✓		✓
Apinjoh, 2015. Cameroon [36]	415	cohort	SP	1 year	✓	✓	✓	✓	
Ndibazza, 2013. Uganda [39]	2289	cohort	SP	5 years	✓				✓
Borgella, 2013. Benin [18]	194	cohort	SP	1 year	✓	✓		✓	✓
Asante, 2013. Ghana [20]	1855	cohort	SP	1 year					✓
Le Port, 2011. Benin [35]	550	cohort	SP	1 year		✓		✓	
Bardaji, 2011.Mozambique [16]	997	RCT	SP vs placebo	1 year		✓	✓	✓	
Schwarz, 2008. Gabon [17]	527	cohort	None	2.5 years		✓			✓
Mutabingwa, 2005. Tanzania [21]	453	cohort	SP	1 year		✓		✓	

[a]Malaria detected in maternal blood by microscopy or PCR,

[b]Placental malaria detected in placental blood by microscopy,

[c]Placental malaria detected in placental tissue by histology

Abbreviations: AL-artemether lumefantrine CQ-chloroquine, IPTp intermittent preventive treatment of malaria in pregnancy, ISTp intermittent screening and treatment of malaria in pregnancy, MQ-mefloquine, RCT randomised controlled trial, SP sulfadoxine-pyrimethamine.

Table 2.2 Association between maternal parasitaemia and malaria risk in infancy stratified by outcome measure

Author, year of publication (ref)	Timing of measurement of maternal parasitaemia	Method used to detect maternal parasitaemia	Exposure groups (n)	Measure of association (95% CI), p-value	Confounders adjusted for
Prevalence of parasitaemia					
Boudova, 2017 [40]	2nd and 3rd trimester	PCR	Unexposed (n=184) Exposed (n=28)	reference OR=1.5 (0.5-4.4), p=0.45	Maternal age, gestation age at delivery, IPTp arm
De Beaudrap, 2016 [37]	2nd and 3rd trimester	Microscopy or RDT	Unexposed (n=626) Exposed (n=198)	reference RR=2.97 (1.37-6.42), p=NR	Gravidity, birth season, haemoglobin genotype, residence
Borgella, 2013 [18]	1st trimester	Microscopy	Unexposed (n=NA) Exposed (n=NA)	reference OR=1.12 (0.23-5.45), p=0.89	Residence near the lake, birth season
	2nd trimester	Microscopy	Unexposed (n=142) Exposed (n=52)	reference OR=0.87 (0.35-2.09), p=0.75	
	3rd trimester	Microscopy	Unexposed (n=121) Exposed (n=73)	reference OR=4.16 (1.64-10.54), p=0.003	
Time to first parasitaemia					
Borgella, 2013 [18]	1st trimester	Microscopy	Unexposed (n=NA) Exposed (n=NA)	reference HR=1.00 (0.42-2.39), p=0.99	Residence near the lake, birth season
	2nd trimester	Microscopy	Unexposed (n=142) Exposed (n=52)	reference HR=1.14 (0.62-2.12), p=0.68	
	3rd trimester	Microscopy	Unexposed (n=121) Exposed (n=73)	reference HR=2.95(1.58-5.50), p=0.001	
Time to first clinical malaria					
Borgella, 2013 [18]	1st trimester	Microscopy	Unexposed (n=NA) Exposed (n=NA)	reference HR=0.97 (0.32-2.92), p=0.95	Residence near the lake, birth season
	2nd trimester	Microscopy	Unexposed (n=142) Exposed (n=52)	reference HR=1.15 (0.58-2.28), p=0.70	
	3rd trimester	Microscopy	Unexposed (n=121) Exposed (n=73)	reference HR=3.19 (1.59-6.38), p=0.001	
Incidence of clinical malaria					
Ndibazza, 2013 [39]	Baseline and delivery	Microscopy	Unexposed (n=2053) Exposed (n=236)	reference HR=1.23 (1.01-1.51), p=0.04	Maternal age, ITN possession, parity, education, social economic status, residence, mother's HIV status

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HR, hazard ratio; IPTp, intermittent preventive treatment of malaria in pregnancy; ITN, insecticide treated net; NA, not applicable, number was imputed; NR, not reported; OR, odds ratio; RDT, rapid diagnostic test; RR, risk ratio; PCR, polymerase chain reaction

Association between maternal parasitaemia and the risk of malaria in infancy

The association between maternal parasitaemia and malaria risk in infancy was assessed in three cohort studies [18, 37, 39] and one RCT of IPTp-SP versus IPTp with chloroquine (CQ) versus CQ prophylaxis conducted in Malawi [40] (Table 2.2). In Malawi, infants born to mothers with malaria parasitaemia detected during the 2nd or 3rd trimester had higher odds of parasitaemia compared to infants born to mothers without parasitaemia during pregnancy, but the difference was not statistically significant [40]. In a cohort study conducted in Uganda, infants born to mothers with any parasitaemia detected by microscopy during pregnancy had a higher risk of parasitaemia during the first year of life compared to infants born to mothers without parasitaemia during pregnancy adjusted for gravidity, birth season, haemoglobin genotype, and residence (risk ratio [RR] 2.97; 95 % confidence interval [CI] 1.37-6.42) [37]. In Benin [18], infants born to mothers having parasitaemia in the 3rd trimester had higher prevalence of parasitaemia (odds ratio [OR] 4.16; 95% CI 1.64-10.54), shorter time to first clinical malaria (hazard ratio [HR] 3.19; 95% CI 1.59-6.38) and shorter time to first parasitaemia (HR 2.95; 95% CI 1.58-5.5) compared to those born to mothers without parasitaemia in the 3rd trimester after adjusting for birth season and residence near the lake (Table 2.2). In the same study, maternal parasitaemia detected during 1st or 2nd trimester was not associated with an increased risk of malaria during infancy [18]. In another cohort study conducted in Uganda, infants born to mothers with malaria parasitaemia at enrolment or at delivery had a higher incidence of clinical malaria during the first five years of life compared to infants born to mothers without parasitaemia (HR 1.23; 95% CI 1.01-1.51) [39].

Association between placental malaria and risk of malaria in infants

Six studies evaluating the association between PM detected by microscopy only and the risk of malaria in infants produced mixed results (Table2.3). The prevalence of parasitaemia was higher in infants born to mothers with PM detected by microscopy compared to those born to mothers without PM (RR 10.42; 95 % CI 2.64-41.10) in a cohort study conducted in Uganda [37] while the prevalence of parasitaemia and clinical malaria tended to be lower in Benin [18] though this was not statistically significant (OR 0.72; 95% CI 0.25-2.11). Compared to infants born to mothers without PM, infants born to mothers with PM detected by microscopy had a shorter time to first parasitaemia in Cameroon [34], Benin [35] and Tanzania [21], and a shorter time to first clinical malaria in Gabon (HR 2.1; 95 % CI 1.2-3.7) [17]. The only study that adjusted for malaria exposure among other confounding factors showed an association between PM detected by microscopy and time to first parasitaemia but only among infants resident in houses with ITNs (HR 2.13; 95 % CI 1.24-3.67) [35]. In another study conducted in Benin, there was no statistically significant

association between PM detected by microscopy and time to first parasitaemia (OR 0.68; 95 % CI 0.34-1.38) or time to first clinical malaria (HR 0.60; 95 % CI 0.28-1.32) in infants [18].

Five studies evaluated associations between PM detected by histology and the risk of malaria in infancy (table 2.4). Histology detected PM was associated with an increase in the odds of clinical malaria in Malawi (OR 3.9; 95% CI 1.2-13.0) [40], Tanzania (OR 4.79; 95% 2.21-10.38) [41], and Mozambique (OR 4.63; 95% CI 2.10-10.24) [16] while one study in Cameroon did not find a statistically significant association between histologically detected PM and prevalence of malaria in infants (OR 0.72; 95% CI 0.40-1.28) [36]. Histologically detected PM was associated with a higher incidence of malaria in Malawi (unadjusted incident rate ratio [IRR] 2.3; 95% CI 1.1-4.8) [40], but this was not observed in Ghana (IRR 0.86; 95% CI 0.54-1.37) [19].

Table 2.3. Association between placental malaria detected by microscopy and the risk of malaria in infancy stratified by outcome.

Author, year of publication (ref)	PM exposure group (n)	Measure of association (95% CI), p-value	Confounders adjusted for
		Prevalence of parasitaemia	
De Beaudrap, 2016 [37]	Unexposed (475) Exposed (15)	reference RR=10.42 (2.64-41.10), p=NR	Gravidity, maternal age, residence, level of education, season, maternal HIV status, ITN use
Borgella, 2013 [18]	Unexposed (154 Exposed (36)	reference OR=0.72 (0.25-2.11), P=0.55	Residence near the lake, birth season
		Time to first parasitaemia	
Tassi Yunga, 2018[a] [34]	No PM (36) PM Lo (18) PM Hi (18)	reference HR=2.6 (1.3-4.8) HR=1.5 (0.7-3.7)	Gravidity, birth season Hb genotype, residence
Borgella, 2013 [18]	Unexposed (154) Exposed (36)	reference HR=0.68 (0.34-1.38), p=0.29	Residence near the lake, birth season
Le Port, 2011 [35]	Unexposed (485) Exposed (59)	reference HR=1.62 (1.08-2.43), p=0.02	Unadjusted
Le Port, 2011 [35]	Unexposed, had ITN (321) Exposed, had ITN (34)	Reference HR= 2.13 (1.24-3.67) p<0.01	Exposure to anopheles, season, antenatal care, severe anaemia
	Unexposed, no ITN (158) Exposed, no ITN (25)	Reference HR=1.18 (0.60-2.33), p=0.62	
Mutabingwa, 2005 [21]	Unexposed (384) Exposed (69)	reference HR=1.41 (1.01-1.99), p=NR	Gravidity, residence, transmission season at birth, and bed net usage
		Time to first clinical malaria	
Borgella, 2013 [18]	Unexposed (154) Exposed (36)	reference HR=0.60 (0.28-1.32), p=0.21	Residence near the lake, birth season
Schwarz, 2008 [17]	Unexposed (477) Exposed (50)	reference HR=2.1 (1.2-3.7), p=NR	Gravidity, residence, birth season, IPTi, bed net use

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HR, hazard ratio; IPTi, intermittent preventive treatment of malaria in infancy; ITN, insecticide treated net; NR, Not reported; OR, odds ratio; PM, placental malaria; RR, risk ratio.

[a]placental malaria detected by microscopy or PCR; PM Lo, placental malaria with <25 infected erythrocytes/µL; PM Hi, placental malaria with >25 infected erythrocytes/µL

Table 2.4 Association between placental malaria detected by histology and the risk of malaria in infancy stratified by outcome.

Author, year of publication (ref)	Placental malaria exposure group (n)	Measure of association (95% CI), p-value	Confounders adjusted for
		Clinical malaria	
Boudova, 2017 [40]	Unexposed (184) Exposed (67)	reference OR=3.9 (1.2-13.0), p=0.03	Maternal age, gestation age at delivery, IPTp arm
Boudova, 2017 [40]	Unexposed (184) Exposed (67)	reference IRR=2.3 (1.1-4.8), p=NR	Unadjusted
Sylvester, 2016 [41]	Unexposed (165) Exposed (41)	reference OR=4.79 (2.21-1O.38), p<0.05	Gravidity, season of birth, infant birth weight, maternal age
Awine, 2016 [19]	Unexposed (484) Exposed (202)	reference IRR=0.86 (0.54-1.37), p=0.52	ITN use, gender, social economic status, living near an irrigated area, infant age, maternal baseline parasitaemia
Apinjoh, 2015 [36]	Unexposed (n=237) Exposed (n=166)	reference OR=0.72 (0.40-1.28), p=0.26	Not indicated
Bardaji, 2011 [16]	Unexposed (424) Past infection (321) Acute infection (42) Chronic infection (82)	reference OR=3.06 (1.94-4.82), p<0.001 OR=4.63 (2.10-10.24), p<0.001 OR=3.95 (2.07-10.24), p<0.001	Residence near the lake, birth season
		Prevalence of parasitaemia	
Boudova, 2017 [40]	Unexposed (184) Exposed (67)	reference OR=2.5 (1.0-6.3), p=0.06	Maternal age, gestation age at delivery, IPTp arm

Abbreviations: CI, confidence interval; IPTp, intermittent preventive treatment of malaria in pregnancy; IRR; incident rate ratio; ITN, insecticide treated net; NR, not reported; OR, odds ratio

Impact of IPTp on the risk of malaria in infants

Four studies evaluated the impact of IPTp on the risk of malaria in infants (Table 2.5). Of these, three were RCTs and one was an observational study where some women received IPTp-SP and others received no IPTp. There was no significant difference in the incidence of malaria among infants born to mothers randomised to IPTp-MQ vs IPTp-SP (IRR 0.95; 95% CI 0.81-1.13) in a multicentre RCT conducted in Benin, Gabon, Tanzania, and Mozambique [38] and among infants born to mothers randomised to intermittent screening and treatment of MiP (ISTp) with artemether-lumefantrine (AL) vs IPTp-SP (IRR 0.94; 95% CI 0.68-1.59) conducted in Ghana [19]. In a cohort study conducted in Ghana, the risk of malaria was higher in infants born to mothers who did not receive IPTp compared to infants born to mothers who received IPTp-SP, but this difference was also not statistically significant [20]. In Mozambique, the odds of clinical malaria in infants born to mothers who were randomised to IPTp-SP were higher, but not statistically significantly so, than in infants born to mothers randomised to placebo (OR 1.28; 95% CI 0.90-1.83) [16].

Table 2.5 Impact of IPTp on the risk of malaria in infancy

Author, year of publication	Randomised Y/N	IPTp arm (n)	Measure of association (95% CI), p-value	Confounders adjusted for
Ruperez, 2016 [38]	Yes	IPTp-SP (1432) IPTP-MQ (2815)	reference IRR=0.95 (0.81-1.13), p=0.60	Country
Awine, 2016 [19]	Yes	IPTp-SP (495) ISTp-AL (493)	reference IRR=0.94 (0.68-1.59), p=0.76	Gender, social economic status, residence, irrigated area, season, ITN use, baseline parasitaemia, maternal haemoglobin
Asante, 2013 [20]	No	IPTp-SP (1755) No IPTp (97)	reference HR=1.23 (0.93-1.59), p=0.15	Unadjusted
Bardaji, 2011 [16]	Yes	Placebo (500) IPTp-SP (497)	reference OR=1.28 (0.90-1.83), p=0.17	Unadjusted

Abbreviations: AL-artemether lumefantrine; CI, confidence interval; IPTp, intermittent preventive treatment of malaria in pregnancy; IRR, incident rate ratio; ISTp intermittent screening and treatment of malaria in pregnancy; ITN, insecticide treated nets; HR, hazard ratio; MQ, mefloquine; SP, sulfadoxine-pyrimethamine

Table 2.6 Assessment of risk of bias for observational studies using the Newcastle Ottawa scale

Author, year of publication (ref)	Selection				comparability		Outcome			Total	Overall risk of bias
	REC	SNEC	ME	DON	AME	AI	AO	FL	CF		
Tassi Yunga, 2018 [34]	*	*	*	*	-	-	*	*	*	7	high
Boudova, 2017 [40]	*	*	*	*	-	*	*	*	-	7	high
Sylvester, 2016 [41]	*	*	*	*	-	-	*	*	-	6	high
De Beaudrap, 2016 [37]	*	*	*	*	-	*	*	*	*	8	high
Apinjoh, 2015 [36]	*	*	*	*	-	-	*	*	-	6	high
Ndibazza, 2013 [39]	*	*	*	*	-	*	*	*	*	8	high
Borgella, 2013 [18]	*	*	*	*	-	*	*	*	*	8	high
Asante, 2013 [20]	*	*	*	*	*	*	*	*	*	9	medium
Le Port, 2011 [35]	*	*	*	*	*	*	*	*	*	9	medium
Schwarz, 2008 [17]	*	*	*	*	-	*	*	*	-	7	high
Mutabingwa, 2005 [21]	*	*	*	*	-	*	*	*	*	8	high

Abbreviations: REC, Representativeness of the exposed cohort; SNEC, selection of the non-exposed cohort, ME, measurement of exposure to malaria during pregnancy; DON, demonstration that the outcome of interest was not present at the start of the study; AME, adjusted for malaria transmission exposure, AI, Adjusted for IPTp or insecticide treated net use; AO, assessment of the outcome, FL, follow-up long enough for outcome to occur; CF; completeness of follow-up. Symbols: ‹-› score of zero, ‹*› score of one.

Assessment of risk of bias in individual studies

Risk of bias in individual studies was assessed using the Newcastle Ottawa scale for observational studies (Table 2.6) and the Cochrane Collaboration tool for RCTs (Table 2.7). One study where IPTp was randomised was assessed using the Newcastle Ottawa Scale for assessing risk of bias in observational studies because the study only assessed an association between MiP (and not IPTp regimens) and the risk of clinical malaria or parasitaemia in the infant [40]. All cohort studies had a representative exposed cohort, selected the non-exposed comparison group adequately and measured malaria exposure during pregnancy, and demonstrated that the outcome was not present at the beginning of follow-up. Most of the studies adjusted for IPTp and ITN use but only two studies [20, 35] adjusted for malaria exposure. In three of the studies, the number of participants lost to follow-up or the reasons for losses to follow-up were not reported [16, 36, 41]. Three studies had >20% losses to follow-up [17, 36, 40]. The overall risk of bias in cohort studies was rated as high in nine studies [17, 18, 21, 34, 36, 37, 39-41] and medium in two studies [20, 35]. In all the RCTs, allocation concealment was adequate, and no trial was stopped before completion. Overall, the risk of bias in all three RCTs was low.

Table 2.7 Assessment of risk of bias in randomised trials comparing the risk of malaria among infants who received different IPTp regimens

Criterion	Studies		
	Ruperez, 2016 [38]	Awine, 2016 [19]	Bardaji, 2011 [16]
Allocation concealment	Yes	Yes	Yes
Trial stopped early	No	No	No
Participants blinded	No	No	Yes
Study staff blinded	No	No	Yes
Infant malaria assessed blinded	Yes	Yes	Yes
Proportion of infants lost to follow-up	972/4247 (22.9%)	NR	NR
Overall risk of bias	Low	Low	Low

Abbreviations: NR, not reported

Discussion

This systematic review assessed evidence evaluating the associations between MiP or IPTp and the risk of malaria infection or illness during infancy. Overall, the available evidence is of insufficient quality to confirm or rule out an association between maternal malaria infection and the risk of malaria during infancy. Most studies had small numbers of exposed infants and failed to control for possible confounding by malaria transmission intensity shared between mothers and their infants. Only one study that examined the association between malaria in pregnancy and the risk of malaria in infants controlled for malaria transmission at the level of the household [35]. In this study, time to first parasitaemia in infants born to mothers with PM was shorter in those who lived in households with ITNs, than in those living in households without ITNs. It is

possible that household use of ITNs reflects underlying malaria transmission intensity, with households exposed to higher transmission more likely to use ITNs, and mothers and infants in such households at higher risk of malaria. However, secondary data analysis of the study did not find an association between PM and the risk of subsequent clinical malaria episodes [42]. This suggests that the effect of PM on the risk of malaria in infants wanes over time.

The majority of the studies included in this review showed an increased risk of clinical malaria or parasitaemia in infants born to mothers with maternal peripheral parasitaemia [37, 39], infants born to mothers with PM detected by microscopy [17, 21, 34, 35, 37] and in infants born to mothers with PM detected by histology [16, 40, 41]. These results could possibly be explained by confounding due to differences in malaria transmission intensity. Infants born to mothers with maternal peripheral parasitaemia during pregnancy or PM at delivery could be at higher risk of clinical malaria or parasitaemia because they live in an environment with higher risk of malaria transmission just like their mothers [43].

Because it would not be ethical and feasible to randomise pregnant women to exposure to MiP, alternative study designs are needed, such as randomising pregnant women to IPTp interventions with different efficacies and comparing the risks of parasitaemia and clinical malaria in the different sets of infants born to mothers who received different IPTp interventions. In this systematic review, three RCTs which evaluated the association between IPTp and the risk of malaria during infancy showed no difference in the risk of malaria among infants born to mothers who received IPTp-MQ vs IPTp-SP [38], ISTp-AL vs IPTp-SP [19], and IPTp-SP vs placebo [16]. These studies were possibly limited by the failure of the alternative intervention to significantly reduce the burden of malaria, especially PM, during pregnancy [44, 45]. The prevalence of PM was not significantly different among mothers randomised to IPTp-MQ (4.6%) compared to IPTp-SP (5.4%, p=0.19) in the multicentre trial [44], and was similar among mothers randomised to IPTp-SP (24.5%) compared to ISTp-AL (24.2%) in the Ghana trial [45]. In the Mozambique trial, the prevalence of any PM detected by histology or microscopy was similar among mothers on IPTp-SP (52%) or placebo (52%) [46]. Although in the same trial, the prevalence of PM detected by microscopy was higher among women on placebo (14%) compared to women on IPTp-SP (7%) [46], the risk of clinical malaria did not differ among infants born to mothers in the two IPTp arms [16]. This could possibly be due to few malaria outcomes during infancy, which limited the power of the study.

There is currently a promising alternative drug combination for IPTp which substantially reduces the burden of malaria during pregnancy including PM compared to SP. Although SP remains the drug recommended by WHO for IPTp [22], its effectiveness is affected by widespread antifolate

resistance [47]. In East Africa, IPTp-DP has been shown to markedly reduce the incidence of clinical malaria and the prevalence of parasitaemia during pregnancy, and the prevalence of PM at delivery compared to IPTp-SP [25, 26, 29]. One study has evaluated the impact of IPTp-DP on malaria during infancy in Uganda. In this randomised controlled trial, which examined infants receiving DP for malaria prevention, the incidence of malaria during the first two years of life was higher in infants born to pregnant women who received IPTp with DP (given monthly) than in those born to women who received IPTp with SP (given every two months). This effect was magnified in female infants [48]. The reason for this finding is unclear but may be due to lower blood levels of piperaquine, which were observed in female infants born to mothers who received IPTp with DP [48]. Lower piperaquine levels have been associated with a higher risk of malaria in children taking DP for malaria prevention [49], but the reason why female infants born to mothers receiving IPTp with DP would have lower levels of piperaquine is unknown.

Several studies have suggested immune tolerance [9, 12, 14, 50] as one of the potential mechanisms for the observed association between MiP and the risk of malaria in infants. Evidence from laboratory studies shows that in-utero exposure to malaria antigens is associated with a bias of foetal immune responses to *P. falciparum* specific [12-14] or non-malaria specific [12, 50] antigens towards anti-inflammatory responses suggesting that exposure to malaria in-utero may not only affect development of malaria-specific immunity in the foetus, but may also affect non-malaria specific immunity. Indeed, one study has reported an increased risk of non-malaria febrile illnesses in infants born to mothers with PM compared to infants born to mothers without PM [51]. Also, PM has been associated with a reduced maternal-foetal transfer of antibodies to *P. falciparum* [52, 53], but this was not associated with an increased risk of malaria in infants [52, 53].

This systematic review had several limitations. Substantial heterogeneity in included studies was found. Studies varied in the duration of follow-up, detection of malaria exposure during pregnancy, and approaches to measuring the outcome in infants and to data analysis. Also, important raw data like the number of malaria episodes in each malaria exposure group during pregnancy was not presented in majority of the studies. Majority of studies included in this systematic review used PM as a proxy measure of MiP, but PM may not be a good proxy measure of MiP because some mothers with peripheral malaria parasitaemia may clear their parasites especially with highly efficacious IPTp drugs. However, the majority of the included studies used IPTp-SP, which is not highly effective at clearing parasites [54]. Only studies published in English were included in this systematic review. However, a search not limiting the language to English did not yield any such studies. The search strategy was also limited to only published studies.

This could have limited the number of studies with null findings which are less likely to be published.

In conclusion, the results of this systematic review suggest that there is insufficient evidence to confirm or exclude a causal association between MiP and the risk of malaria during infancy. Also, evidence on the impact of IPTp on the risk of malaria in infancy is inconclusive. There is need to better understand the association between MiP and malaria in infants in order to minimise the effects of MiP on malaria during infancy. Future studies of new IPTp interventions should consider not only evaluating the impact of IPTp on birth outcomes but also the potential impact of the intervention on the risk of clinical malaria or parasitaemia in infancy. This could have important policy implications on the choice of future drugs for IPTp.

Received 20 May 2019 Accepted 28 August 2019

Published online 03 September 2019

References

1. Dellicour S, Tatem AJ, Guerra CA, Snow RW, ter Kuile FO: **Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study.** *PLoS Med* 2010, **7:**e1000221.

2. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, Newman RD: **Epidemiology and burden of malaria in pregnancy.** *Lancet Infect Dis* 2007, **7:**93-104.

3. Omer SA, Idress HE, Adam I, Abdelrahim M, Noureldein AN, Abdelrazig AM, Elhassan MO, Sulaiman SM: **Placental malaria and its effect on pregnancy outcomes in Sudanese women from Blue Nile State.** *Malar J* 2017, **16:**374.

4. Guyatt HL, Snow RW: **Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa.** *Clin Microbiol Rev* 2004, **17:**760-769, table of contents.

5. Guyatt HL, Snow RW: **The epidemiology and burden of *Plasmodium falciparum*-related anemia among pregnant women in sub-Saharan Africa.** *Am J Trop Med Hyg* 2001, **64:**36-44.

6. Moore KA, Simpson JA, Scoullar MJL, McGready R, Fowkes FJI: **Quantification of the association between malaria in pregnancy and stillbirth: a systematic review and meta-analysis.** *Lancet Glob Health* 2017, **5:**e1101-e1112.

7. Steketee RW, Wirima JJ, Hightower AW, Slutsker L, Heymann DL, Breman JG: **The effect of malaria and malaria prevention in pregnancy on offspring birthweight, prematurity, and intrauterine growth retardation in rural Malawi.** *Am J Trop Med Hyg* 1996, **55:**33-41.

8. Dauby N, Goetghebuer T, Kollmann TR, Levy J, Marchant A: **Uninfected but not unaffected: chronic maternal infections during pregnancy, fetal immunity, and susceptibility to postnatal infections.** *Lancet Infect Dis* 2012, **12:**330-340.

9. Brustoski K, Moller U, Kramer M, Petelski A, Brenner S, Palmer DR, Bongartz M, Kremsner PG, Luty AJ, Krzych U: **IFN-gamma and IL-10 mediate parasite-specific immune responses of cord blood cells induced by pregnancy-associated *Plasmodium falciparum* malaria.** *J Immunol* 2005, **174:**1738-1745.

10. Malhotra I, Mungai P, Muchiri E, Ouma J, Sharma S, Kazura JW, King CL: **Distinct Th1- and Th2-Type prenatal cytokine responses to *Plasmodium falciparum* erythrocyte invasion ligands.** *Infect Immun* 2005, **73:**3462-3470.

11. Metenou S, Suguitan Jr AL, Long C, Leke RGF, Taylor DW: **Fetal immune responses to *Plasmodium falciparum* antigens in a malaria-endemic region of Cameroon.** *J Immunol* 2007, **178:**2770-2777.

12. Bisseye C, van der Sande M, Morgan WD, Holder AA, Pinder M, Ismaili J: ***Plasmodium falciparum* infection of the placenta impacts on the T helper type 1 (Th1)/Th2 balance of neonatal T cells through CD4(+)CD25(+) forkhead box P3(+) regulatory T cells and interleukin-10.** *Clin Exp Immunol* 2009, **158:**287-293.

13. Ismaili J, Van Der Sande M, Holland MJ, Sambou I, Keita S, Allsopp C, Ota MO, McAdam KPWJ, Pinder M: ***Plasmodium falciparum* infection of the placenta affects newborn immune responses.** *Clin Exp Immunol* 2003, **133:**414-421.

14. Brustoski K, Moller U, Kramer M, Hartgers FC, Kremsner PG, Krzych U, Luty AJ: **Reduced cord blood immune effector-cell responsiveness mediated by CD4+ cells induced in utero as a consequence of placental *Plasmodium falciparum* infection.** *J Infect Dis* 2006, **193:**146-154.

15. Engelmann I, Santamaria A, Kremsner PG, Luty AJ: **Activation status of cord blood gamma delta T cells reflects in utero exposure to *Plasmodium falciparum* antigen.** *J Infect Dis* 2005, **191:**1612-1622.

16. Bardaji A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, Mabunda S, Alonso PL, Menendez C: **Impact of malaria at the end of pregnancy on infant mortality and morbidity.** *J Infect Dis* 2011, **203:**691-699.

17. Schwarz NG, Adegnika AA, Breitling LP, Gabor J, Agnandji ST, Newman RD, Lell B, Issifou S, Yazdanbakhsh M, Luty AJ, et al: **Placental malaria increases malaria risk in the first 30 months of life.** *Clin Infect Dis* 2008, **47:**1017-1025.

18. Borgella S, Fievet N, Huynh BT, Ibitokou S, Hounguevou G, Affedjou J, Sagbo JC, Houngbegnon P, Guezo-Mevo B, Massougbodji A, et al: **Impact of pregnancy-associated malaria on infant malaria infection in southern Benin.** *PLoS One* 2013, **8:**e80624.

19. Awine T, Belko MM, Oduro AR, Oyakhirome S, Tagbor H, Chandramohan D, Milligan P, Cairns M, Greenwood B, Williams JE: **The risk of malaria in Ghanaian infants born to women managed in pregnancy with intermittent screening and treatment for malaria or intermittent preventive treatment with sulfadoxine/pyrimethamine.** *Malar J* 2016, **15:**46.

20. Asante KP, Owusu-Agyei S, Cairns M, Dodoo D, Boamah EA, Gyasi R, Adjei G, Gyan B, Agyeman-Budu A, Dodoo T, et al: **Placental malaria and the risk of malaria in infants in a high malaria transmission area in ghana: a prospective cohort study.** *J Infect Dis* 2013, **208:**1504-1513.

21. Mutabingwa TK, Bolla MC, Li JL, Domingo GJ, Li X, Fried M, Duffy PE: **Maternal malaria and gravidity interact to modify infant susceptibility to malaria.** *PLoS Med* 2005, **2:**e407.

22. **WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP).** [http://www.who.int/malaria/publications/atoz/iptp-sp-updated-policy-brief-24jan2014.pdf]

23. Naidoo I, Roper C: **Drug resistance maps to guide intermittent preventive treatment of malaria in African infants.** *Parasitology* 2011, **138:**1469-1479.

24. van Eijk AM, Larsen DA, Kayentao K, Koshy G, Slaughter DEC, Roper C, Okell LC, Desai M, Gutman J, Khairallah C, et al: **Effect of *Plasmodium falciparum* sulfadoxine-pyrimethamine resistance on the effectiveness of intermittent preventive therapy for malaria in pregnancy in Africa: a systematic review and meta-analysis.** *The Lancet Infectious Diseases* 2019.

25. Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, Ruel T, Opira B, Ochokoru H, Ategeka J, Nayebare P, et al: **Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in pregnancy: a double-blind, randomised, controlled, superiority trial.** *Lancet* 2019.

26. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, Opira B, Olwoch P, Ategeka J, Nayebare P, et al: **Dihydroartemisinin-piperaquine for the prevention of malaria in pregnancy.** *N Engl J Med* 2016, **374:**928-939.

27. Broen K, Brustoski K, Engelmann I, Luty AJ: **Placental *Plasmodium falciparum* infection: causes and consequences of in utero sensitization to parasite antigens.** *Mol Biochem Parasitol* 2007, **151:**1-8.

28. Garner P, Gulmezoglu AM: **Drugs for preventing malaria in pregnant women.** *Cochrane Database Syst Rev* 2006**:**Cd000169.

29. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, Ouma P, Were V, Laserson K, Katana A, et al: **Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with**

sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial. *Lancet* 2015, **386:**2507-2519.

30. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA: **Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation.** *BMJ* 2015, **349:**g7647.

31. **Malaria in Pregnancy Library** [http://library.mip-consortium.org/]

32. **The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomized studies in metaanalyses** [http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp]

33. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA: **The Cochrane Collaboration's tool for assessing risk of bias in randomised trials.** *BMJ* 2011, **343:**d5928.

34. Tassi Yunga S, Fouda GG, Sama G, Ngu JB, Leke RGF, Taylor DW: **Increased susceptibility to *Plasmodium falciparum* in infants is associated with low, not high, placental malaria parasitemia.** *Sci Rep* 2018, **8:**169.

35. Le Port A, Watier L, Cottrell G, Ouedraogo S, Dechavanne C, Pierrat C, Rachas A, Bouscaillou J, Bouraima A, Massougbodji A, et al: **Infections in infants during the first 12 months of life: role of placental malaria and environmental factors.** *PLoS ONE [Electronic Resource]* 2011, **6:**e27516.

36. Apinjoh TO, Anchang-Kimbi JK, Mugri RN, Njua-Yafi C, Tata RB, Chi HF, Tangoh DA, Loh BT, Achidi EA: **Determinants of infant susceptibility to malaria during the first year of life in South Western cameroon.** *Open Forum Infect Dis* 2015, **2:**ofv012.

37. De Beaudrap P, Turyakira E, Nabasumba C, Tumwebaze B, Piola P, Boum Ii Y, McGready R: **Timing of malaria in pregnancy and impact on infant growth and morbidity: a cohort study in Uganda.** *Malar J* 2016, **15:**92.

38. Ruperez M, Gonzalez R, Mombo-Ngoma G, Kabanywanyi AM, Sevene E, Ouedraogo S, Kakolwa MA, Vala A, Accrombessi M, Briand V, et al: **Mortality, morbidity, and developmental outcomes in infants born to women who received either mefloquine or sulfadoxine-pyrimethamine as intermittent preventive treatment of malaria in pregnancy: a cohort study.** *PLoS Medicine / Public Library of Science* 2016, **13:**e1001964.

39. Ndibazza J, Webb EL, Lule S, Mpairwe H, Akello M, Oduru G, Kizza M, Akurut H, Muhangi L, Magnussen P, et al: **Associations between maternal helminth and malaria infections in pregnancy and clinical malaria in the offspring: a birth cohort in entebbe, Uganda.** *J Infect Dis* 2013, **208:**2007-2016.

40. Boudova S, Divala T, Mungwira R, Mawindo P, Tomoka T, Laufer MK: **Placental but not peripheral *Plasmodium falciparum* Infection during pregnancy is associated with increased risk of malaria in infancy.** *J Infect Dis* 2017, **216:**732-735.

41. Sylvester B, Gasarasi DB, Aboud S, Tarimo D, Massawe S, Mpembeni R, Swedberg G: **Prenatal exposure to *Plasmodium falciparum* increases frequency and shortens time from birth to first clinical malaria episodes during the first two years of life: prospective birth cohort study.** *Malar J* 2016, **15:**379.

42. Bouaziz O, Courtin D, Cottrell G, Milet J, Nuel G, Garcia A: **Is placental malaria a long term risk factor for mild malaria attack in infancy? Revisiting a paradigm.** *Clin Infect Dis* 2017.

43. Cairns M, Gosling R, Chandramohan D: **Placental malaria increases malaria risk in the first 30 months of life: not causal.** *Clin Infect Dis* 2009, **48:**497-498; author reply 498-499.

44. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, Kakolwa MA, Abdulla S, Accrombessi M, Aponte JJ, Akerey-Diop D, Basra A, Briand V, et al: **Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-negative women: a multicentre randomized controlled trial.** *PLoS Med* 2014, **11:**e1001733.

45. Tagbor H, Cairns M, Bojang K, Coulibaly SO, Kayentao K, Williams J, Abubakar I, Akor F, Mohammed K, Bationo R, et al: **A non-inferiority, individually randomized trial of intermittent screening and treatment versus intermittent preventive treatment in the control of malaria in pregnancy.** *PLoS One* 2015, **10:**e0132247.

46. Menendez C, Bardaji A, Sigauque B, Romagosa C, Sanz S, Serra-Casas E, Macete E, Berenguera A, David C, Dobano C, et al: **A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic.** *PLoS One* 2008, **3:**e1934.

47. Naidoo I, Roper C: **Mapping 'partially resistant', 'fully resistant', and 'super resistant' malaria.** *Trends in Parasitology* 2013, **29:**505-515.

48. Jagannathan P, Kakuru A, Okiring J, Muhindo MK, Natureeba P, Nakalembe M, Opira B, Olwoch P, Nankya F, Ssewanyana I, et al: **Dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria during pregnancy and risk of malaria in early childhood: a randomized controlled trial.** *PLoS Med* 2018, **15:**e1002606.

49. Sundell K, Jagannathan P, Huang L, Bigira V, Kapisi J, Kakuru MM, Savic R, Kamya MR, Dorsey G, Aweeka F: **Variable piperaquine exposure significantly impacts protective efficacy of monthly dihydroartemisinin-piperaquine for the prevention of malaria in Ugandan children.** *Malar J* 2015, **14:**368.

50. Gbedande K, Varani S, Ibitokou S, Houngbegnon P, Borgella S, Nouatin O, Ezinmegnon S, Adeothy AL, Cottrell G, Massougbodji A, et al: **Malaria modifies neonatal and early-life toll-like receptor cytokine responses.** *Infect Immun* 2013, **81:**2686-2696.

51. Rachas A, Le Port A, Cottrell G, Guerra J, Choudat I, Bouscaillou J, Massougbodji A, Garcia A: **Placental malaria is associated with increased risk of nonmalaria infection during the first 18 months of life in a Beninese population.** *Clin Infect Dis* 2012, **55:**672-678.

52. Dechavanne C, Cottrell G, Garcia A, Migot-Nabias F: **Placental malaria: decreased transfer of maternal antibodies directed to *Plasmodium falciparum* and impact on the incidence of febrile infections in infants.** *PLoS ONE [Electronic Resource]* 2015, **10:**e0145464.

53. Moro L, Bardaji A, Nhampossa T, Mandomando I, Serra-Casas E, Sigauque B, Cistero P, Chauhan VS, Chitnis CE, Ordi J, et al: **Malaria and HIV infection in Mozambican pregnant women are associated with reduced transfer of antimalarial antibodies to their newborns.** *J Infect Dis* 2015, **211:**1004-1014.

54. Desai M, Gutman J, Taylor SM, Wiegand RE, Khairallah C, Kayentao K, Ouma P, Coulibaly SO, Kalilani L, Mace KE, et al: **Impact of sulfadoxine-pyrimethamine resistance on effectiveness of intermittent preventive therapy for malaria in pregnancy at clearing infections and preventing low birth Weight.** *Clin Infect Dis* 2016, **62:**323-333.

2.4 Updates to the systematic review literature

In this section, I review more recent literature including systematic reviews and original research articles reporting on the impact of MiP and IPTp on the risk of malaria in infants published since my systematic review was completed. Considering this additional literature, the conclusions of my systematic review are still valid.

2.1.4 Other systematic reviews on the impact of malaria in pregnancy on the risk of malaria in infants

One additional systematic review and meta-analysis was published in March 2020 [1]. The objective of this new systematic review was to quantify the risk of malaria in children born to mothers who had malaria during pregnancy. In this review, the authors included cohort studies, case control studies, and studies with follow-up period of at least 3 months, and conducted meta-analyses. This new review differed from my review in 3 important ways: 1) only prospective cohort studies were included in my analysis while the new systematic review included case-control, and retrospective cohort studies in addition to prospective cohort studies; 2) only studies with a follow-up period of at least 6 months were considered in my review while studies with shorter follow-up periods were included in this new systematic review; and 3) a meta-analysis was not conducted in my review due to substantial heterogeneity in studies while in this new review, a meta-analysis was conducted. The new review identified 19 published articles, two of which were from the same birth cohort study [2, 3] conducted in Tanzania, which reported on the impact of MiP on the risk of malaria in children. Thirteen of the studies included in this new systematic review were also included in my review.

The five of the six additional studies which were not included in my review did not show evidence of a significant association between MiP and malaria in children. The birth cohort conducted in Tanzania [2] reported a non-significant higher risk of severe malaria during 4 years of follow-up in children born to secundi or multigravidae mothers with PM (HR 1.86, 95% CI 0.92-3.77, p=0.08) and those born to primigravidae mothers with PM (HR 1.12, 95% CI 0.44-2.82, p=0.81) compared to those born to primigravidae mothers without PM. In a cohort study conducted in Kenya [4], there was no difference in the risk of parasitaemia detected by microscopy during one year of follow-up among infants born to mothers with PM (15.6%) compared to those born to mothers without PM (14.7%). A cohort study conducted in Cameroon [5, 6] reported a higher prevalence of parasitaemia from 4-18 months of age, among children born to mothers with PM detected by microscopy, compared to those born to mothers without PM [6], although no measure of effect was reported. In a randomised trial conducted in Malawi,

there was no difference in the odds of parasitaemia during the first 3 months of life among infants born to mothers with PM detected by microscopy compared to those born to mothers without PM (unadjusted OR 1.1, 95% CI 0.7-1.9, p=0.71) [7]. The sixth study, conducted in Kenya by Malhotra et al [8] reported a higher risk of malaria parasitaemia during the first 3 years of life in infants born to mothers with parasitaemia or PM, compared to those born to mothers without maternal parasitaemia or PM (RR 1.61, 95% CI 1.10-2.43, p=0.024), after adjusting for repeated measures, time, and location. Moreover, these infants were found to be immune tolerant following stimulation of cord blood mononuclear cells with malaria antigens suggesting that in-utero malaria exposure may lead to bias of immune responses to malaria towards tolerance which may increase the risk of malaria in later life.

In this new review, meta-analyses were conducted despite the substantial heterogeneity between studies as demonstrated by the Higgin's I-squared values of >50% [1]. The authors pooled infant results if the exposure was maternal parasitaemia or PM (regardless of how PM was detected) and the effect measure in the studies included was identical. The results of the meta-analysis showed that maternal parasitaemia or PM were associated with non-significant higher odds of parasitaemia (pooled odds ratio [OR], 1.94, 95% confidence interval [CI] 0.93-4.07), and a significantly higher risk of first parasitaemia (pooled hazard ratio [HR] 1.46, 95% CI 1.07-2.00). Similarly, maternal parasitaemia or PM were associated with higher odds of clinical malaria (pooled OR 2.82, 95% CI 1.82-4.38), and a higher risk of first clinical malaria (pooled HR 1.31, 95% CI 0.96-1.79), although this was not significant. The authors concluded that overall, MiP was associated with a higher risk of malaria in children but acknowledged that most of the included studies failed to control for the potential confounding effects of malaria transmission intensity, which was a major limitation.

2.2.4 Other studies on the impact of malaria in pregnancy or IPTp on the risk of malaria in infants

I repeated the literature search using the same strategy applied in my systematic review and found two additional birth cohort studies, one conducted in Benin, published in December 2018 [9], and another from Burkina Faso, published in September 2018 [10], which examined the impact of maternal parasitaemia or PM on the risk of malaria in infants. The birth cohort conducted in Benin [9], was part of a multicentre randomised controlled trial in which HIV-uninfected pregnant women were randomised to IPTp with SP versus MQ (15mg/kg given in one day) versus split dose MQ (15mg/Kg given over two days) conducted in Benin, Gabon, Mozambique, and Tanzania [11] and were followed-up to delivery. A subset of infants born to mothers in Benin were then followed-up to one year and the odds of parasitaemia or clinical

malaria during the first year of life were compared among infants born to mothers with parasitaemia or PM at delivery detected by microscopy and those born to mothers with no parasitaemia or PM at delivery adjusting for factors including small for gestation age, maternal age, social economic status, and exposure to mosquitoes. The authors found that compared to no parasitaemia or PM at delivery, parasitaemia or PM at delivery was associated with higher odds of parasitaemia among infants during the first 6 months (aOR 2.18, 95% CI 0.95-4.95, p=0.07), and between 6-12 months of life (aOR 1.31, 95% CI 0.62-2.78, p=0.48) [9], but the differences were not significant. Parasitaemia or PM at delivery was also associated with higher odds of clinical malaria during the first 6 months of life (aOR 1.62, 95% CI 0.84-3.58, p=0.13), and lower odds of clinical malaria between 6-12 months of life (aOR 0.91, 95% CI 0.39-2.14, p=0.83) compared to no parasitaemia or PM at delivery, but the differences were not statistically significant.

The second birth cohort in Burkina Faso [10] was part of the COSMIC study, a multicentre cluster randomised trial conducted in Burkina Faso, Benin, and the Gambia, where villages were randomised to one of two treatment arms, 1) a combination of monthly community-scheduled screening and treatment of malaria in pregnancy (CSST) with rapid diagnostic tests and artemether-lumefantrine (AL) plus IPTp with SP (CSST/IPTp-SP), and 2) monthly IPTp-SP alone [12]. Pregnant women from the participating villages were then followed-up to delivery. Infants born to mothers in Burkina Faso were then followed-up from birth to one year of age; the risk of malaria in infancy was compared between infants born to mothers with PM detected by histology and those born to mothers without PM. The authors found that PM was associated with a lower risk of clinical malaria among infants between 0-6 months of age, but a higher risk of malaria among infants between 6-12 months of age, although effect estimates (hazard ratios) were not reported [10].

Another publication from the same Burkina Faso birth cohort described above, was also retrieved. In this publication, the effect of in-utero malaria exposure and the impact of monthly CSST/IPTp-SP on the risk of first malaria episode among infants were assessed [13]. Compared to infants born to uninfected mothers (no maternal or PM), after adjusting for gravidity, low birth weight, and birth season, infants born to mothers with peripheral parasitaemia (adjusted HR [aHR] 1.62, 95% CI 1.12-2.32, p=0.009) and those born to mothers with past PM (aHR 1.42, 95% CI 1.06-1.91, p=0.02) had a higher risk of first malaria episode. Infants born to mothers with active PM had a higher risk of first malaria episode compared to those born to uninfected mothers, but this finding was not significant (aHR 1.30, 95% CI 0.89-1.91, p=0.18). Monthly CSST/IPTp-SP was associated with a modest 12% lower risk of first malaria episode among infants compared to IPTp-SP alone [13] but the difference was not statistically significant (aHR

0.88, 95% CI 0.73-1.07, p=0.21). There was no association between monthly CSST/IPTp-SP and the incidence of malaria in infants (adjusted IRR 0.95, 95% CI 0.82-1.10, p=0.46).

2.3.4 Summary

In summary, the recent systematic review and meta-analysis [1], and the additional COSMIC publications identified [10, 13] showed that maternal peripheral parasitaemia and PM were associated with an increased risk of malaria in infants. Like my review, the recent review and meta-analysis was largely limited by the heterogeneity of included studies, most of which were also limited by the failure to adjust for potential confounding by malaria exposure. The birth cohort in Burkina Faso did not find that adding community scheduled screening and treatment with RDTs and AL to IPTp reduced the risk of malaria in infants born over IPTp-SP alone. Considering this, the conclusions of my systematic review remain valid. Evidence to confirm or exclude a causal association between MiP, or the prevention of MiP, and the risk of malaria in infancy is lacking.

2.5 References

1. Park S, Nixon CE, Miller O, Choi NK, Kurtis JD, Friedman JF, Michelow IC: **Impact of malaria in pregnancy on risk of malaria in young children: systematic review and meta-analyses.** *J Infect Dis* 2020.

2. Goncalves BP, Huang CY, Morrison R, Holte S, Kabyemela E, Prevots DR, Fried M, Duffy PE: **Parasite burden and severity of malaria in Tanzanian children.** *N Engl J Med* 2014, **370:**1799-1808.

3. Mutabingwa TK, Bolla MC, Li JL, Domingo GJ, Li X, Fried M, Duffy PE: **Maternal malaria and gravidity interact to modify infant susceptibility to malaria.** *PLoS Med* 2005, **2:**e407.

4. Bonner PC, Zhou Z, Mirel LB, Ayisi JG, Shi YP, van Eijk AM, Otieno JA, Nahlen BL, Steketee RW, Udhayakumar V: **Placental malaria diminishes development of antibody responses to *Plasmodium falciparum* epitopes in infants residing in an area of western Kenya where *P. falciparum* is endemic.** *Clin Diagn Lab Immunol* 2005, **12:**375-379.

5. Cot M, Le Hesran JY, Staalsoe T, Fievet N, Hviid L, Deloron P: **Maternally transmitted antibodies to pregnancy-associated variant antigens on the surface of erythrocytes infected with *Plasmodium falciparum*: relation to child susceptibility to malaria.** *Am J Epidemiol* 2003, **157:**203-209.

6. Le Hesran JY, Cot M, Personne P, Fievet N, Dubois B, Beyeme M, Boudin C, Deloron P: **Maternal placental infection with *Plasmodium falciparum* and malaria morbidity during the first 2 years of life.** *Am J Epidemiol* 1997, **146:**826-831.

7. Slutsker L, Khoromana CO, Hightower AW, Macheso A, Wirima JJ, Breman JG, Heymann DL, Steketee RW: **Malaria infection in infancy in rural Malawi.** *Am J Trop Med Hyg* 1996, **55:**71-76.

8. Malhotra I, Dent A, Mungai P, Wamachi A, Ouma JH, Narum DL, Muchiri E, Tisch DJ, King CL: **Can prenatal malaria exposure produce an immune tolerant phenotype? A prospective birth cohort study in Kenya.** *PLoS Med* 2009, **6:**e1000116.

9. Agbota G, Accrombessi M, Cottrell G, Martin-Prével Y, Milet J, Ouédraogo S, Courtin D, Massougbodji A, Garcia A, Cot M, Briand V: **Increased risk of malaria during the first year of life in small-for-gestational-age infants: a longitudinal study in Benin.** *J Infect Dis* 2019, **219:**1642-1651.

10. Natama HM, Moncunill G, Rovira-Vallbona E, Sanz H, Sorgho H, Aguilar R, Coulibaly-Traore M, Some MA, Scott S, Valea I, et al: **Modulation of innate immune responses at birth by prenatal malaria exposure and association with malaria risk during the first year of life.** *BMC Med* 2018, **16:**198.

11. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, Kakolwa MA, Abdulla S, Accrombessi M, Aponte JJ, Akerey-Diop D, Basra A, Briand V, et al: **Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-negative women: a multicentre randomized controlled trial.** *PLoS Med* 2014, **11:**e1001733.

12. Scott S, Mens PF, Tinto H, Nahum A, Ruizendaal E, Pagnoni F, Grietens KP, Kendall L, Bojang K, Schallig H, D'Alessandro U: **Community-based scheduled screening and treatment of malaria in pregnancy for improved maternal and infant health in The Gambia, Burkina Faso and Benin: study protocol for a randomized controlled trial.** *Trials* 2014, **15:**340.

13. Natama HM, Rovira-Vallbona E, Sorgho H, Some MA, Traore-Coulibaly M, Scott S, Zango SH, Sawadogo O, Zongo SC, Valea I, et al: **Additional screening and treatment of malaria during prregnancy provides further protection against malaria and nonmalarial fevers during the first year of life.** *J Infect Dis* 2018, **217:**1967-1976.

CHAPTER 3 OBJECTIVES, AND METHODS

3.1 Introduction

In this chapter, the thesis objectives, the respective hypotheses, and the study methods used to answer the thesis questions and objectives are presented, including the study design, study participants, follow-up of study participants, laboratory procedures, study outcomes and statistical analyses performed.

3.2 Study objectives and hypotheses

1) To compare the incidence of malaria in infants during the first year of life among infants born to mothers with PM detected by microscopy, LAMP, or histology and those born to mothers without PM.

Hypothesis: Infants born to mothers with PM detected by microscopy, LAMP, or histology will have a higher incidence of malaria in the first year of life compared to infants born to mothers without PM.

2) To compare the incidence of malaria during the first year of life in infants born to mothers who were randomised to receive monthly IPTp-DP versus monthly IPTp-SP.

Hypothesis: Infants born to mothers randomised to receive monthly IPTp-DP will have a lower incidence of malaria during the first year of life compared to infants born to mothers randomised to receive monthly IPTp-SP.

3) To evaluate the effect of IPTp and PM on cord blood levels of IgG antibodies to *P. falciparum* malaria antigens.

Hypothesis 1: Infants born to mothers with PM detected by microscopy, LAMP, or histology will have lower mean blood levels of IgG antibodies to P. falciparum antigens at birth compared to those born to mothers without PM.

Hypothesis 2: Compared to infants born to mothers randomised to monthly IPTp-SP, infants born to mothers randomised to monthly IPTp-DP will have higher mean blood levels of IgG antibodies to *P. falciparum* antigens at birth.

3.3 Methods

3.1.3 Study design

This study was part of a completed double-blind, randomised, controlled trial (RCT) (ClinicalTrials.gov: NCT02793622) of monthly IPTp with DP or SP for the prevention of malaria in HIV-uninfected pregnant women and their infants. The trial involved two phases: the pregnancy and infant phases. The pregnancy phase of the study involved enrolment and follow-up of pregnant women through delivery. The main objective of the pregnancy phase of the study was to compare the risk of a composite adverse birth outcome (preterm delivery [< 37 weeks of gestation], low birth weight [< 2500g], or small for gestation age [< 10^{th} percentile based on reference population]) among mothers who received IPTp-DP and mothers who received IPTp-SP, and the findings, which are not the subject of this thesis, were published [1]. The infant phase, which is the focus of this thesis, involved follow up of all live births to mothers who took part in the RCT, from birth to one year of age. The study was conducted from September 2016 to December 2018.

3.2.3 Study area

This study was conducted in Busia district, Uganda. Busia district, an area of high malaria endemicity, is located on the border between Kenya and Uganda and is bordered to the south by Lake Victoria (Figure 3.1).

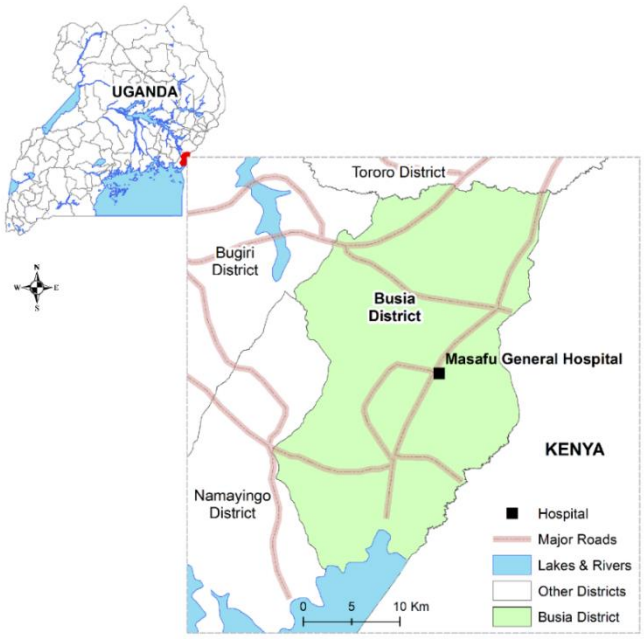

Figure 3.1 A map of Uganda showing location of Busia district, the study area

Study participants

Participants of the pregnancy phase of the study were pregnant women of all gravidities. Pregnant women were enrolled if they fulfilled the following criteria: 1) a viable intrauterine pregnancy confirmed by ultrasound; 2) estimated gestational age between 12-20 weeks; 3) confirmed HIV- uninfected status by rapid test; 4) 16 years of age or older; 5) residing in Busia District, Uganda; 6) agreement to come to the study clinic for any febrile episode or other illness and avoid medications given outside the study protocol; 7) plan to deliver in the hospital; 8) no history of a serious adverse event related to DP or SP; and 9) provision of informed consent for themselves and their unborn infants. Participants of the infant phase of the study were live births of pregnant women who were enrolled in the trial.

3.3.3 Randomisation and study drug administration

At enrolment, pregnant women were randomised to receive IPTp-DP or IPTp-SP in a ratio of 1:1. A randomisation list consisting of permuted blocks of 4 or 8 was computer generated by a staff member not involved in patient care. Treatment assignment was done by a study pharmacist not involved in patient care. Study drugs were initiated at 16 (for women enrolled at ≤16 weeks and one day of gestation) or 20 (for those enrolled at >16 weeks and one day of gestation) weeks of gestation and were administered every 4 weeks thereafter. A dose of DP consisted of 3 tablets of 40 mg of dihydroartemisinin and 320 mg of piperaquine (Duo-Cotexin, Holley-Cotec, Beijing, China) given once a day for 3 days. A dose of SP consisted of 3 tablets of 500 mg of sulfadoxine and 25 mg of pyrimethamine (Kamsidar, Kampala Pharmaceutical Industries), given as a single dose. In addition, participants randomised to DP received SP placebos, while those randomised to SP received DP placebos every 4 weeks to achieve blinding. All doses of study drugs were prepackaged by a study pharmacist and dispensed by study nurses blinded to the study participant's treatment regimen. For DP or DP placebo, all 1st daily doses were administered directly observed in the clinic. The 2nd and 3rd daily doses were dispensed to the mother for self-administration at home. Administration of all SP and SP placebo doses were directly observed in the study clinic.

3.4.3 Follow-up of pregnant women

At enrolment, a clinical assessment including history and clinical examination was conducted. Baseline laboratory tests included a complete blood count (CBC) and the detection of malaria parasites by microscopy and quantitative PCR (qPCR). All enrolled women were given a long-lasting insecticide treated net, and a baseline household survey to assess household

characteristics including the house structure and household possessions among others [2], was conducted. Women were followed up for all their medical care in a dedicated study clinic located in Masafu General Hospital and were reviewed routinely every 4 weeks. Routine assessments included a standardised history and clinical assessment, study drug administration, and collection of blood samples (thick blood smear and a capillary blood sample) for detection of malaria parasites by microscopy or qPCR. Routine blood smears were read later and were not used for the management of pregnant women. At any study visit, pregnant women who were febrile (with a history of fever or a tympanic temperature ≥ 38.0˚C) had a finger prick done for an urgent thick blood smear for malaria diagnosis. Pregnant women diagnosed with malaria were treated using either AL or artesunate according to the Uganda Ministry of Health guidelines. Women were encouraged to deliver at Masafu General Hospital. At delivery, babies were weighed on standard scales. Maternal, cord, and placental blood, for making dried blood spots and thick blood smears for detecting malaria parasites, and placental tissue samples for detecting PM by histology were collected. Post-partum visits were conducted in the study clinic at 1, and 6 weeks after delivery.

3.5.3 Follow-up of infants

All infants born alive were followed up for all their medical care in a dedicated study clinic, open daily from 8:00 am - 5:00 pm. Parents/guardians were encouraged to bring their infants to the study clinic for routine assessments and any time they were ill. Routine assessments were conducted at 1, 4, 6 and 8 weeks of age, and thereafter every 4 weeks until infants reached 52 weeks of age. The 4 weekly routine assessments included a standardised history taking, a clinical exam, and collection of thick blood smears for detection of malaria parasitaemia. At 12, 28, and 52 weeks, blood was drawn for measuring haemoglobin (Hb) level using either a CBC or a portable spectrophotometer (HemoCue, Angholm, Sweden), and plasma sample was stored. Infants who were febrile (with a history of fever in the past 24 hours or a tympanic temperature ≥ 38.0˚C) at any clinic visit had an urgent thick blood smear done for malaria parasites and were diagnosed with malaria if the urgent smear was positive. Febrile infants with negative urgent thick blood smears were treated for non-malaria febrile illness. Infants who were not febrile but had a malaria positive routine thick blood smear were not treated for malaria in accordance with local guidelines.

3.6.3 Malaria diagnosis and treatment

Study participants with fever and a positive thick blood smear were diagnosed with malaria and another finger prick done for a thin blood smear for identifying parasite species and urgent Hb

level measurement using a portable spectrophotometer (HemoCue). Malaria episodes were classified as uncomplicated, complicated or treatment failure. Uncomplicated malaria was diagnosed in participants with fever and without danger signs (≤ 2 convulsions in 24 hours, vomiting everything, inability to sit or stand, unable to breastfeed or drink, and lethargy) or severe malaria. Complicated malaria was diagnosed in participants with danger signs or severe malaria. Treatment failure was defined as a malaria episode preceded by another malaria episode in the last 14 days. A new malaria episode was defined as a malaria episode not preceded by another malaria episode in the last 14 days. All infants diagnosed with malaria were treated according to the Uganda Ministry of Health guidelines. New episodes of uncomplicated malaria were treated with artemether lumefantrine (AL). Children with complicated malaria were treated with artesunate and those with a new episode of malaria who were less than 4 months of age or <5kg of weight, were treated with quinine. Those diagnosed with treatment failures within 14 days following treatment with AL were treated with quinine. Patients with treatment failure within 14 days following treatment with quinine or artesunate were treated with quinine plus clindamycin or artesunate.

3.7.3 Premature withdrawal of study participants

Participants were prematurely withdrawn if they meet any of the following criteria: 1) movement out of study area, 2) inability to be located for > 60 consecutive days, 3) withdrawal of informed consent, 4) inability to comply with the study schedule and procedures, 5) at the discretion of the site investigator if the study is not in the best interest of the participant, or 6) subject or parent/guardian judged by the site investigator to be at significant risk of failing to comply with the study protocol as to cause them harm or seriously interfere with the validity of study results.

3.8.3 Laboratory methods

Thick blood smears were stained with 2% Giemsa for half-hour and examined under a light microscope by trained laboratory technicians who were blinded to the mother's study treatment arm. A thick blood smear was declared negative if 100 high power fields were examined without showing any asexual parasites. Thin smears were used for parasite species identification. For quality control, all blood smears were examined by two independent laboratory technicians and smears with discrepant results were examined by a 3rd laboratory technician.

Quantitative polymerase chain reaction (qPCR) for detection of *P. falciparum* malaria parasites was done as previously described [3], on 200 µL of maternal blood samples collected at

enrolment, and during routine assessment. DNA was extracted from whole blood using PureLink DNA extraction kit. Parasites were then detected in the extracted sample by detecting the *var*ATS gene. The LAMP assay for detection of malaria parasites from dried placental blood spots collected at delivery was performed using Loopamp™ MALARIA Pan Detection Kit (Eiken Chemical Company, Japan) as previously described [4]. In brief, parasite DNA was extracted from placental dried blood spots using chelex extraction method and amplified in reaction tubes, which were incubated at 65.0˚C for 40 minutes. Reaction tubes were then visualized in a fluorescence visualization unit. Positive samples emitted green light.

Placental biopsy specimens were assessed for histological evidence of PM as previously described [5]. In brief, placental biopsy specimens were embedded in paraffin wax, sectioned into 3 µM slices using a rotary microtome, fixed to glass slides, and dehydrated in sequential ethanol baths. Separate slides were stained in 0.1% hematoxylin and 1% eosin for 5 and 1 min, respectively, or in 2% Giemsa for 30 minutes and then microscopically examined. The presence of intervillous parasite-infected erythrocytes and of pigment in monocyte/macrophages or fibrin was noted. The quantity of malaria pigment deposited in fibrin, expressed as the proportion of high-power fields (HPF) with malaria pigment deposition in fibrin was assessed as described by Muelenbach's et al [6].

3.9.3 Measurement of *P. falciparum* IgG antibodies

Measurement of antibodies to *P. falciparum* was done on stored maternal and cord plasma samples collected at delivery, and on infant plasma samples. All plasma samples were stored at -80°C. Levels of antibodies to *P. falciparum* antigens (including antigens associated with long term responses such as MSP 1 and 2, AMA1, and antigens with shorter half-lives such EBA-175, Rh-5 were measured using a multiplex antibody bead assay. Luminex bead conjugation was performed as previously described [7]. 50 µl thawed plasma, in duplicate (1/200 dilution) were co-incubated with microsphere mixtures on a 96-well plate for one hour, washed, stained, and incubated with a secondary antibody, then washed and read by the MAGPIX system. Antibody levels were expressed in arbitrary units (AUs), calculated by dividing the median fluorescence intensity (MFI) of the sample by the MFI plus 3 standard deviations (SD) of samples from North Americans never exposed to malaria. Positive control samples from individuals with known antibodies to these antigens were placed on each plate. Standard curves were generated through serial dilutions of the positive control pool. The qualitative nature of the immune response was investigated in infants in each arm by measuring IgG (total & isotype responses) specific responses and the avidity of antibodies to these antigens.

3.10.3 Study outcomes

The primary outcome for objective 1 and 2 was the incidence of malaria in the first 12 months of life defined as the number of incident episodes of malaria per person-year at risk. An incident episode of malaria was defined as the presence of fever (history of fever in the last 24 hours or a tympanic temperature ≥ 38.0˚C) with malaria positive thick blood smear not preceded by another episode in the last 14 days. The primary outcome for objective 3 was the mean levels of specific *P. falciparum* IgG antibodies. Secondary outcomes are presented in Table 3.1 below.

Table 3.1 Secondary outcomes

Secondary outcome	Definition
Time to first malaria episode	Time from birth to first episode of malaria
Incidence of complicated malaria	Number of malaria episodes with danger signs (convulsions, vomiting everything, lethargy) or with severe malaria according to the WHO definition
Prevalence of malaria parasitaemia	Proportion routine thick blood smears with malaria parasites detected by microscopy
Prevalence of anaemia	Proportion of haemoglobin measurements done at 12, 28, and 52 weeks that were <10 g/dL or <8 g/dL
Incidence of hospital admissions	Number of all cause hospital admissions
Incidence of non-malaria febrile illnesses	Number of episodes of fever not preceded by another episode in the last 7 days (history of fever in the last 24 hours or a tympanic temperature ≥ 38.0˚C) with a malaria negative blood smear
Infant mortality	All cause deaths occurring within 12 months of life

3.11.3 Sample size and power calculation

An independent estimation of the sample size of the infant phase of the study was not done. Instead, the sample size of the infant phase of the study was determined by the estimated sample size of the pregnancy phase of the study and the resulting live births. The sample size of the pregnancy phase of the study was estimated to be 782 pregnant women and it was assumed that 95% would result into live births in which the primary outcome of the pregnancy phase of the study was assessed [1]. With the resulting live births, to test the hypothesis that compared to IPTp-SP, IPTp-DP is associated with a lower incidence of malaria among infants during the first 12 months of life, it was assumed that malaria incidence would be 3-5 episodes per person-year among infants born to mothers on IPTp-SP (using data from a prior study conducted in the adjacent district of Tororo [8]) and that 5% of infants would be lost to follow-up. With these assumptions, the study had 80% power at 5% level of significance (two tailed), to detect an 18-

23% difference in the incidence of malaria among infants born to mothers on IPTp-DP compared to those born to mothers on IPTp-SP.

3.12.3 Data analysis

Data were double entered and checked by two trained independent data officers into an Access database. Data analyses were conducted using Stata version 14.2. Follow-up period started from the birth date and ended on the date the infant was one year old or the date the infant was prematurely withdrawn.

Maternal characteristics at enrolment, during follow-up, and at delivery, and infant characteristics at birth were compared among infants born to mothers who received monthly IPTp-DP and those born to mothers who received monthly IPTp-SP. Characteristics with simple proportions were compared using a chi-squared test, continuous outcomes were analysed using a t-test, incidence measures were analysed using negative binomial regression, and repeated prevalence measures were analysed using generalized estimating equations with robust standard errors. For objective 1, PM detected by microscopy, LAMP or histology, was categorised as follows: no PM (absence of parasites or pigment); active PM (parasites detected in placental blood or tissue by microscopy LAMP or histology, with or without pigment [9, 10]); mild-moderate past PM (>0-20% HPFs with pigment without parasites); or severe past PM (>20% HPFs with pigment without parasites). Placental inflammation was not considered in the grading PM. Comparisons among infants in different PM categories taking the no PM category as the reference group were made adjusting for maternal IPTp arm, gravidity (categorised as primigravidae, secundigravidae, or multigravidae), housing construction type (modern or traditional), and maternal parasitaemia status detected by microscopy or qPCR at enrolment. For objective 2, comparisons were made between infants born to mothers who received monthly IPTp-DP and those born to mothers who received monthly IPTp-SP. Because the association between IPTp and the risk of malaria in children had been previously reported to vary with infant sex [11], assessments for effect modification by infant sex in the analyses were made a priori. In all comparisons, negative binomial regression models were used to compare incidence measures, Cox proportional hazards models were used for comparisons of first malaria episode in infants, and generalised estimating equations with robust standard errors were used to compare prevalence outcomes measured repeatedly during infancy.

To estimate the proportion of the effect of IPTp-DP compared to IPTp-SP on the incidence of malaria in infancy was mediated through prevention of PM, mediation analysis using inverse odds ratio weighting (IOW) [12] was conducted. The exposure, mediator, and outcome of interest were maternal IPTp regimen, PM, and the incidence of malaria during infancy,

respectively (figure 3.2). Since IPTp was randomised, it was assumed that there was no potential confounding in the association between IPTp and PM, and between IPTp and malaria incidence in infants. Maternal parasitaemia at enrolment, gravidity, and housing construction type, were considered as potential confounders in the association between PM and malaria incidence in infants. Three models were used to estimate the total, direct, and indirect (mediated) effects. In the first model, treatment weights were generated using logistic regression, by specifying treatment as a function of the mediator and potential confounders in the association between PM and outcome. The predicted probabilities were obtained from the first model and used to generate inverse odds as treatment weights for each maternal-infant pair. In the second model, negative binomial regression was used to estimate the effect of IPTp DP versus SP on malaria incidence in infants, first for all infants and then stratified by infant sex, this estimated the total effect. In the third model, negative binomial regression was used to estimate the direct effect by modelling the association between IPTp and incidence of malaria in infancy weighted for the inverse odds predicted in the first model above. The mediated effect was then calculated by subtracting the direct effect from the total effect. 95% confidence intervals were then calculated by bootstrapping using 1,000 simulations. The proportion of the effect mediated by PM was calculated using the formula, $[\ln(IRR_{\text{indirect effect}})])/[\ln(IRR_{\text{total effect}})]*100$, where IRR=incidence rate ratio.

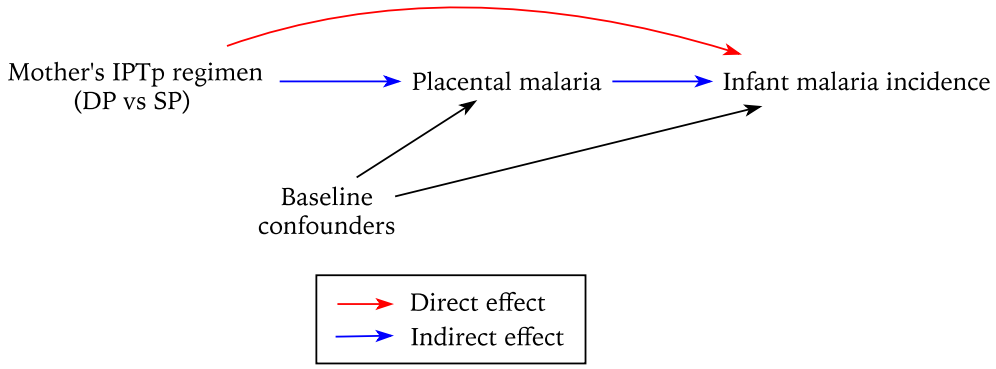

Figure 3.2 Causal diagram showing how the relationship between intermittent preventive treatment of malaria in pregnancy and infant malaria, is mediated by placental malaria

For objective 3, log transformed mean levels of different *P. falciparum* IgG antibodies were calculated separately for maternal, and cord blood. Using a t-test, log transformed mean levels of *P. falciparum* IgG antibodies in maternal blood were compared among mothers who received monthly IPTp-DP and those who received monthly IPTp-SP, and among mothers in different PM categories. Using linear regression models, log transformed mean levels of IgG *P. falciparum* antibodies in cord blood were compared among infants born to mothers who received IPTp-DP and those born to mothers who received IPTp-SP, and among infants born to mothers in

different PM categories. Adjustments for potential confounders including maternal gravidity, maternal parasitaemia at enrolment, and housing construction type, were made. Potential associations between levels *P. falciparum* IgG antibodies in cord blood and the incidence of malaria were evaluated using negative binomial regression.

In all analyses, two-tailed p-values of < 0.05 were considered statistically significant.

3.13.3 Ethical considerations

Ethical approval was obtained from Makerere University school of Biomedical Sciences Ethics Committee, the Uganda National Council of Science and Technology, the University of California San Francisco Research Ethics committee, and the Stanford University Institutional Review Board. In addition, the PhD part of the study was approved by the London School of Hygiene and Tropical Medicine Ethics Committee (Appendices D-H). Written informed consent was obtained from all participants before enrolment.

3.14.3 Summary of the main trial

The pregnancy phase of the study took place between September 2016 to December 2017 and the results have been published [1]. In summary, 782 HIV-uninfected pregnant women residing in Busia district, were enrolled between September 2016 and May 2017 and were randomised to monthly IPTp-DP (N=391) or monthly IPTp-SP (N=391). The homes of enrolled study participants were well- distributed across Busia district (Figure 3.3). A total of 687 (88%) women, 338 receiving IPTp-SP and 349 receiving IPTp-DP, were followed-up through delivery resulting in 678 live births between December 2016 and December 2017. At enrolment, 82% of the enrolled mothers had parasitaemia detected by microscopy or qPCR. During pregnancy, monthly IPTp-DP was associated with a 96% (95% CI 88%-99%, p=0.001) lower risk of clinical malaria and 98% (95% CI 96%-99%, p<0.0001) lower risk parasitaemia detected by microscopy, compared to IPTp-SP. At delivery, IPTp-DP was associated with a 97% (75%-99%, p=0.0009), 90% (80%-96%, p<0.0001) and 54% (44%-62%, p<0.0001) lower risk of placental malaria detected by microscopy, LAMP, or histology, respectively, compared to IPTp-SP. However, there was no evidence that infants born mothers receiving IPTp-DP had a lower risk of adverse birth outcomes compared to infants born to mothers receiving IPTp-SP [1]. Results of the infant phase of the study are presented in this thesis in chapters 4, 5, and 6.

Trial registration

Trial registration: ClinicalTrials.gov, NCT02793622. Registered 8 June 2016, https://clinicaltrials.gov/ct2/show/NCT02793622

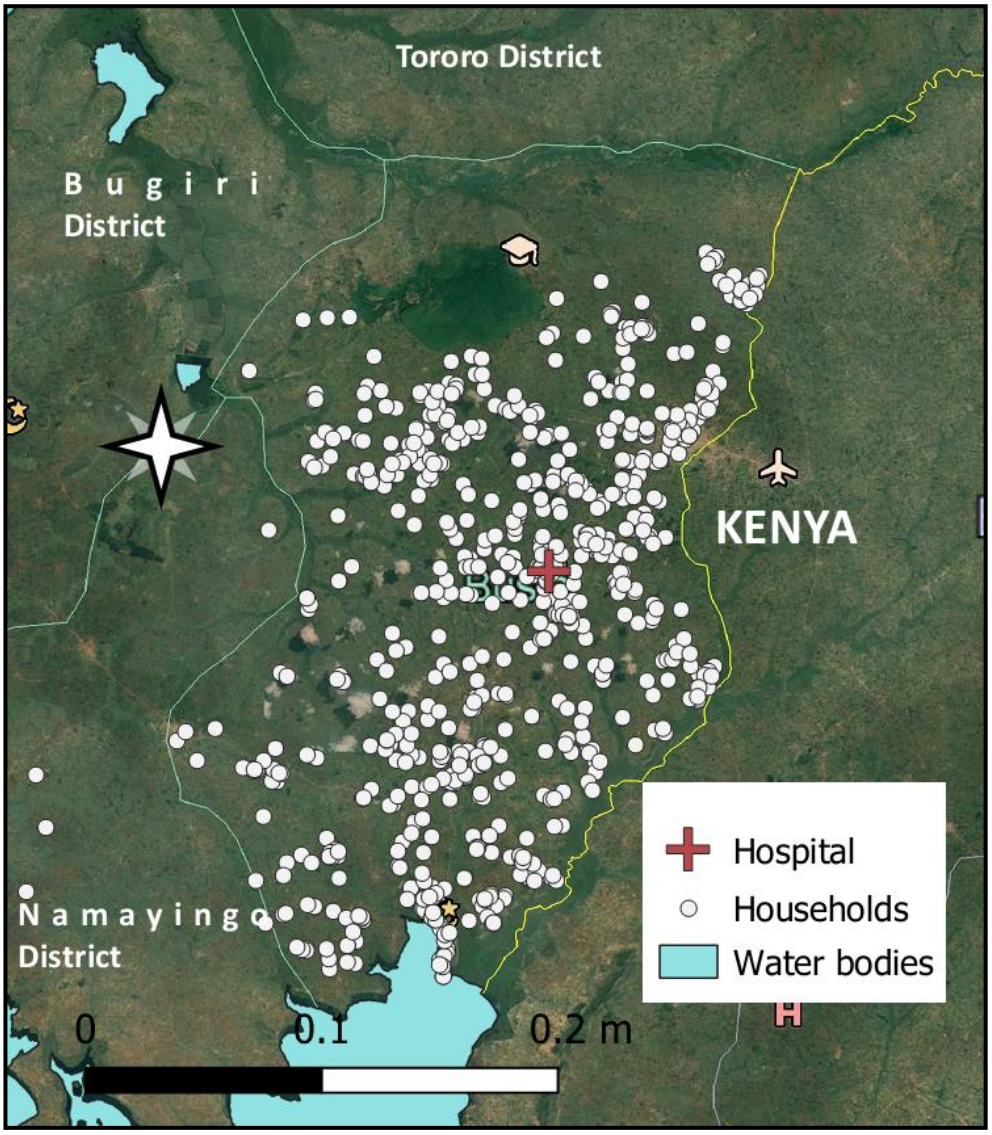

Figure 3.3 A map showing location of homes of enrolled study participants

3.4 References

1. Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, Ruel T, Opira B, Ochokoru H, Ategeka J, Nayebare P, et al: **Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in pregnancy: a double-blind, randomised, controlled, superiority trial.** *Lancet* 2019, **393:**1428-1439.

2. Okiring J, Olwoch P, Kakuru A, Okou J, Ochokoru H, Ochieng TA, Kajubi R, Kamya MR, Dorsey G, Tusting LS: **Household and maternal risk factors for malaria in pregnancy in a highly endemic area of Uganda: a prospective cohort study.** *Malar J* 2019, **18:**144.

3. Katrak S, Murphy M, Nayebare P, Rek J, Smith M, Arinaitwe E, Nankabirwa JI, Kamya M, Dorsey G, Rosenthal PJ, Greenhouse B: **Performance of loop-mediated isothermal amplification for the identification of submicroscopic *Plasmodium falciparum* infection in Uganda.** *Am J Trop Med Hyg* 2017, **97:**1777-1781.

4. Hopkins H, Gonzalez IJ, Polley SD, Angutoko P, Ategeka J, Asiimwe C, Agaba B, Kyabayinze DJ, Sutherland CJ, Perkins MD, Bell D: **Highly sensitive detection of malaria parasitemia in a malaria-endemic setting: performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda.** *J Infect Dis* 2013, **208:**645-652.

5. Natureeba P, Ades V, Luwedde F, Mwesigwa J, Plenty A, Okong P, Charlebois ED, Clark TD, Nzarubara B, Havlir DV, et al: **Lopinavir/ritonavir-based antiretroviral treatment (ART) versus efavirenz-based ART for the prevention of malaria among HIV-infected pregnant women.** *J Infect Dis* 2014, **210:**1938-1945.

6. Muehlenbachs A, Fried M, McGready R, Harrington Whitney E, Mutabingwa Theonest K, Nosten F, Duffy Patrick E: **A novel histological grading scheme for placental malaria applied in areas of high and low malaria transmission.** *The Journal of Infectious Diseases* 2010, **202:**1608-1616.

7. Ambrosino E, Dumoulin C, Orlandi-Pradines E, Remoue F, Toure-Balde A, Tall A, Sarr JB, Poinsignon A, Sokhna C, Puget K, et al: **A multiplex assay for the simultaneous detection of antibodies against 15 *Plasmodium falciparum* and *Anopheles gambiae* saliva antigens.** *Malar J* 2010, **9:**317.

8. Jagannathan P, Muhindo MK, Kakuru A, Arinaitwe E, Greenhouse B, Tappero J, Rosenthal PJ, Kaharuza F, Kamya MR, Dorsey G: **Increasing incidence of malaria in children**

despite insecticide-treated bed nets and prompt anti-malarial therapy in Tororo, Uganda. *Malar J* 2012, **11:**435.

9. Bardaji A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, Mabunda S, Alonso PL, Menendez C: **Impact of malaria at the end of pregnancy on infant mortality and morbidity.** *J Infect Dis* 2011, **203:**691-699.

10. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, Hirt R, Cardesa A, Alonso PL: **Placental pathology in malaria: a histological, immunohistochemical, and quantitative study.** *Hum Pathol* 2000, **31:**85-93.

11. Jagannathan P, Kakuru A, Okiring J, Muhindo MK, Natureeba P, Nakalembe M, Opira B, Olwoch P, Nankya F, Ssewanyana I, et al: **Dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria during pregnancy and risk of malaria in early childhood: a randomized controlled trial.** *PLoS Med* 2018, **15:**e1002606.

12. Nguyen QC, Osypuk TL, Schmidt NM, Glymour MM, Tchetgen Tchetgen EJ: **Practical guidance for conducting mediation analysis with multiple mediators using inverse odds ratio weighting.** *Am J Epidemiol* 2015, **181:**349-356.

CHAPTER 4 ASSOCIATION BETWEEN PLACENTAL MALARIA AND INCIDENCE OF MALARIA DURING INFANCY

4.1 Chapter Introduction

This chapter addresses Objective 1, to compare the incidence of malaria in infants during the first year of life among infants born to mothers with PM detected by histology and infants born to mothers without PM detected by histology. This manuscript presents results of secondary analysis of data from a birth cohort of infants born to HIV-uninfected mothers who took part in a double-blind, randomised, controlled trial of monthly IPTp with DP versus SP. The manuscript was published in the Malaria Journal. At the end of the manuscript, a table of baseline characteristics of study participants stratified by infant sex is presented.

4.2 Research paper

Below is the research paper cover sheet for the manuscript, followed by the manuscript, tables, and figures.

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1602857	**Title**	Dr
First Name(s)	Abel		
Surname/Family Name	Kakuru		
Thesis Title	Impact of malaria in pregnancy and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants		
Primary Supervisor	Sarah G. staedke		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Malaria Journal		
When was the work published?	December 2020		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	Not applicable		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	

Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I had input in study conception, participated in obtaining study IRB approvals, coordinated and participated in data collection, conducted data analysis, and wrote the first draft of the manuscript

SECTION E

Student Signature	[redacted]
Date	04 February 2021

Supervisor Signature	[redacted]
Date	19 Feb 2021

Kakuru et al. Malar J (2020) 19:449
https://doi.org/10.1186/s12936-020-03522-z

Malaria Journal

RESEARCH Open Access

Infant sex modifies associations between placental malaria and risk of malaria in infancy

Abel Kakuru[1,2*], Michelle E. Roh[3], Richard Kajubi[2], Teddy Ochieng[2], John Ategeka[2], Harriet Ochokoru[2], Miriam Nakalembe[4], Tamara D. Clark[5], Theodore Ruel[6], Sarah G. Staedke[1], Daniel Chandramohan[1], Diane V. Havlir[5], Moses R. Kamya[7], Grant Dorsey[5] and Prasanna Jagannathan[8*]

Abstract

Background

Placental malaria (PM) has been associated with a higher risk of malaria during infancy. However, it is unclear whether this association is causal, and is modified by infant sex, and whether intermittent preventive treatment in pregnancy (IPTp) can reduce infant malaria by preventing PM.

Methods

Data from a birth cohort of 656 infants born to HIV-uninfected mothers randomised to IPTp with dihydroartemisinin-piperaquine (DP) or sulfadoxine-pyrimethamine (SP) was analysed. PM was categorised as no PM, active PM (presence of parasites), mild-moderate past PM (>0-20% high powered fields [HPFs] with pigment), or severe past PM (>20% HPFs with pigment). The association between PM and incidence of malaria in infants stratified by infant sex was examined. Causal mediation analysis was used to test whether IPTp can impact infant malaria incidence via preventing PM.

Results

There were 1088 malaria episodes diagnosed among infants during 596.6 person years of follow-up. Compared to infants born to mothers with no PM, the incidence of malaria was higher among infants born to mothers with active PM (adjusted incidence rate ratio [aIRR] 1.30, 95% CI 1.00-1.71, p=0.05) and those born to mothers with severe past PM (aIRR 1.28, 95% CI 0.89-1.83, p=0.18), but the differences were not statistically significant. However, when stratifying by

infant sex, compared to no PM, severe past PM was associated a higher malaria incidence in male (aIRR 2.17, 95% CI 1.45-3.25, p<0.001), but not female infants (aIRR 0.74, 95% CI 0.46-1.20, p=0.22). There were no significant associations between active PM or mild-moderate past PM and malaria incidence in male or female infants. Male infants born to mothers given IPTp with DP had significantly less malaria in infancy than males born to mothers given SP, and 89.7% of this effect was mediated through prevention of PM.

Conclusion

PM may have more severe consequences for male infants, and interventions which reduce PM could mitigate these sex-specific adverse outcomes. More research is needed to better understand this sex-bias between PM and infant malaria risk.

Keywords Placental malaria, pregnancy, infants, *Plasmodium falciparum*

---

*Correspondence: akakuru@idrc-uganda.org; prasj@stanford.edu

1 London School of Hygiene and Tropical Medicine, London, UK

8 Department of Medicine, Stanford University, Stanford, USA

Full list of author information is available at the end of the article

Background

Plasmodium falciparum remains a major public health problem affecting mainly pregnant women and young children. In pregnant women infected with *P. falciparum*, parasitized erythrocytes sequester in the placenta, resulting in placental malaria (PM), which is characterised by placental inflammation, parasite infiltration, and deposition of malaria pigment, a product of digestion of haemoglobin by *P. falciparum* [1]. Placental malaria is associated with adverse effects such as preterm delivery, low birth weight, stillbirth, and neonatal mortality [2-4]. Despite the use of preventive measures including insecticide treated nets and intermittent preventive therapy during pregnancy (IPTp), the burden of PM among pregnant women living in high malaria transmission settings remains high [5].

There is evidence that PM may impact infants after birth [6-8]. Several observational studies have reported associations between PM and increased risks of malaria, non-malaria febrile illnesses, and anaemia in infancy, possibly due to immune tolerance induced by *in-utero* exposure to malaria antigens [9-13]. However, most of these studies defined PM as the detection of malaria parasites in placental blood by microscopy which has limited sensitivity and does not account for past placental infections characterised by the presence of malaria pigment [14]. Furthermore, the severity of malaria pigment deposition in the placenta was recently shown to be strongly predictive of adverse birth outcomes including low birth weight and preterm birth [15]. Whether the severity of malaria pigment deposition in the placenta is also associated with the risk of malaria in infancy is unknown.

A recent double-blind randomised controlled trial compared the incidence of malaria during infancy among infants born to mothers who received monthly IPTp with dihydroartemisinin-piperaquine (DP) versus sulfadoxine-pyrimethamine (SP). In this trial, infants born to mothers receiving IPTp-DP had a lower malaria incidence compared to infants born to mothers receiving IPTp-SP. However, this association was observed in male, but not female, infants [16]. To further evaluate the association between PM and the incidence of malaria in infants, a secondary and mediation analysis of these data was carried out to examine how much of the previously observed associations between IPTp and the risk of malaria in infants is mediated through prevention of PM.

Methods

Study design, setting, and participants

Data were collected from a birth cohort of infants born to HIV-uninfected pregnant women enrolled in a randomized controlled trial of monthly IPTp with DP vs SP (Trial registration, ClinicalTrials.gov; NCT02793622) conducted in Busia district, Uganda, an area of perennial high malaria transmission intensity. Details of the study have been previously reported [5, 16, 17]. Pregnant women were enrolled at 12-20 weeks of gestation and followed through delivery. At delivery, placental blood and tissue samples were collected. Following delivery, all live births were followed up to 12 months of age. Mothers were encouraged to bring their infants to a dedicated study clinic open every day for all their medical care. Routine assessments were conducted every 4 weeks for clinical assessment and collection of blood smears for the detection of parasites by microscopy. Infants presenting with a history of fever in the past 24 hours or a documented tympanic temperature $\geq$ 38.0˚C had a thick blood smear collected for detection of malaria parasites and those diagnosed with malaria were treated according to the Uganda Ministry of Health guidelines. Non-malarial illnesses were treated according to the integrated management of childhood illnesses guidelines. At 12, 28, and 52 weeks of age, blood was collected for haemoglobin measurement.

Laboratory methods

Thick blood smears were stained with 2% Giemsa and read by microscopists [5]. Haemoglobin measurements were made using a spectrophotometer (Hemocue, Angelholm, Sweden). Malaria parasites were detected in placental blood by microscopy and loop-mediated isothermal amplification (LAMP) [18]. Placental biopsy specimens were embedded in paraffin wax, sectioned using a rotary microtome, fixed on glass slides, and dehydrated in sequential ethanol baths [19]. Separate slides were stained in 0.1% hematoxylin and 1% eosin for 5 and 1 min, respectively, or in 2% Giemsa for 30 minutes and examined for presence of intervillous parasite-infected erythrocytes and malaria pigment by two independent readers. The proportion of high-power fields (HPF) with malaria pigment deposition in fibrin was analysed as described [20].

Study outcomes

The primary outcome was the incidence of malaria from birth to 12 months of age. An incident episode of malaria was defined as the presence of fever (history of fever in the past 24 hours or a tympanic temperature ≥ 38·0˚C) with a positive thick blood smear not preceded by another malaria episode in the last 14 days. Secondary outcomes included time to first episode of malaria; incidence of complicated malaria (malaria with danger signs or meeting standardised criteria for severe malaria), all-cause hospitalisations; and non-malarial febrile illnesses; prevalence of malaria parasitaemia during routine visits and anaemia (haemoglobin <10g/dL); and infant mortality.

Statistical methods

Data were double entered and verified in Microsoft Access, and statistical analyses conducted using Stata (14.2) including all live births with placental histology results. Follow-up began at birth and ended at 12 months of age or premature study withdrawal. The primary exposure variable, PM, was categorised as follows: no PM (absence of parasites or pigment); active PM (parasites detected in placental blood or tissue by microscopy LAMP or histology, with or without pigment [9, 21]); or past PM (presence of pigment, without parasites). Relationships between past PM and infant malaria incidence were initially evaluated by considering the proportion of HPF with pigment as a continuous variable. Given the non-linear nature of this relationship, past PM was further characterised into 2 groups based on best fit associations with the outcome variable: mild-moderate past PM (>0-20% HPFs with pigment without parasites); or severe past PM (>20% HPFs with pigment without parasites). Analyses were stratified by infant sex a priori. Associations between PM and the incidence of malaria were performed using negative binomial regression and adjusted for maternal parasitaemia status at enrolment, IPTp arm, gravidity, housing construction type, and clustering for twin gestation. The cumulative risk of any first episode of malaria was compared using a Cox proportional hazards model. For secondary outcomes, incident and repeated prevalence measures were compared using negative binomial regression model and generalized estimating equations with robust standard errors, respectively. Mediation analysis, using inverse odds weighting (IOW) [22], was used to estimate what proportion of the reported effect between maternal IPTp regimen and malaria incidence in infants [16] was mediated through preventing PM [Supplementary Appendix]. In brief, we used three models to conduct IOW mediation analyses. The first model used logistic regression to model treatment given mediator (PM) and mediator-outcome confounders. Predicted probabilities obtained from this model were then used to calculate treatment IOWs for each mother-infant pair. The second and third models used negative binomial regression to model the outcome given treatment with and without weights, respectively. The treatment coefficient from the model with weights estimated the direct effect, which was then subtracted from the treatment coefficient of the model without weights (total effect) to estimate the mediated effect. Bias-corrected 95% confidence intervals (CIs) were computed using bootstrapping. The proportion mediated by PM was calculated by dividing the mediated effect by the total effect. In all analyses, p-values of <0·05 were considered statistically significant.

Results

Study profile and characteristics of study participants

Between September 2016 and May 2017, 782 HIV-uninfected pregnant women were enrolled and randomised to receive either IPTp-SP or IPTp-DP, and 687 (87.9%) followed through delivery resulting in 678 live births (Figure 4.1) [16]. A total of 656 infants with placental histology results were included in the analyses. Mean maternal age at enrolment was 24 years and 23.5% infants were born to primigravida mothers. During pregnancy, the incidence of malaria and prevalence of malaria parasitaemia were significantly lower among women randomised to IPTp-DP compared to those randomised to IPTp-SP. At delivery, women randomised to IPTp-DP had a significantly lower prevalence of active PM (2.1% versus 21.7% p<0.001) and severe past PM (1.8% versus 12.2%, p<0.001) compared to those randomised to IPTp-SP (Table 4.1).

Association between placental malaria and the incidence of malaria in infants

Overall, 1088 incident episodes of malaria were diagnosed over 596.6 person years of follow-up (1.82 episodes per person year). Each 1% increase in the proportion of HPF with pigment deposition in fibrin was associated with a higher incidence of malaria in infants but the difference was not statistically significant (adjusted incidence rate ratio [aIRR] 1.98, 95% CI 0.76-5.20, p=0.16). However, on stratifying by infant sex, significant interaction was observed (P-interaction [p_{int}]= 0.03). Among male infants, a 1% increase in HPF with pigment deposition in fibrin was associated with over 5 times higher incidence of malaria (aIRR 5.20, 95% CI 1.70-15.94, p=0.004). No significant difference in the incidence of malaria was observed in female infants (aIRR 0.53, 95% CI 0.13-2.11 p=0.37). Considering PM as a categorical variable, compared to infants born to mothers with no PM, the incidence of malaria was higher among infants born to mothers with active PM (aIRR 1.30, 95% CI 1.00-1.71, p=0.05) and those born to mothers with severe past PM (aIRR 1.28, 95% CI 0.89-1.83, p=0.18, Table 4.2), but the differences were not statistically significant. However, effect modification by infant sex was also observed in the association between categories of PM and the incidence of malaria in infants (p_{int} = 0.02). Compared to no PM, severe past PM was associated with a higher incidence of malaria among male infants (aIRR 2.17, 95% CI 1.45-3.25, p<0.001), but not among female infants (Table 4.2) There were no significant associations between active PM or mild-moderate past PM and the incidence of malaria in male or female infants. There was no significant difference in the overall rate of first malaria episode among infants born to mothers with active PM (adjusted hazard ratio [aHR] 1.03, 95% CI 0.72-1.47, p=0.88), mild-moderate past PM (aHR 0.92, 95% CI 0.71-1.18, p=0.51), or severe past PM (aHR 1.18, 95% CI 0.75-1.86, p=0.47), compared to those born to mothers with no PM. However, the association between PM and the rate of first malaria episode was also modified by infant sex. Compared to no PM, severe past PM was associated with a significantly higher rate of first malaria episode among male infants (aHR 1.99, 95% CI 1.04-3.81, p=0.04; Figure 4.2); no significant association was observed in female infants.

Association between placental malaria and other malaria outcomes in infants

The incidence of complicated malaria was non-significantly higher among infants born to mothers with active PM (aIRR 1.72, 95% CI 0.81-3.66, p=0.16), and severe past PM (aIRR 2.44, 95% CI 0.93-6.37, p=0.07; Table 4.3). The prevalence of parasitaemia detected during routine visits was also similar among infants born to mothers with active or past PM compared to infants born to mothers with no PM (Table 4.3). However, effect modification by infant sex was also observed in the association between PM and other malaria outcomes. Among male infants, severe past PM was associated with a nearly four-fold higher incidence of complicated malaria (aIRR 3.88, 95% CI 1.14-13.03, p=0.03) and a more than two-fold higher prevalence of malaria parasitaemia during routine visits (risk ratio 2.24, 95% CI 1.34-3.74, p=0.002) compared to no PM. No significant associations were observed among female infants (Table 4.3).

Association between placental malaria and non-malarial outcomes in infancy

There were 16 deaths (2.4% of infants) and 25 all-cause hospitalisations during follow-up. Severe past PM was associated with a non-significant higher incidence of all-cause hospitalisations among infants (aIRR 2.90, 95% CI 0.59-14.44, p=0.19; Table 4.4) compared to no PM. There were no significant differences in the incidence of non-malaria febrile illnesses and prevalence of anaemia during routine visits among infants born to mothers in different PM categories compared to no PM (Table 4.4).

Does prevention of PM explain the difference in infant malaria incidence between IPTp-DP and IPTp-SP?

Male infants born to mothers who received IPTp-DP have been previously reported to have a lower incidence of malaria in infancy compared to male infants whose mothers received IPTp-SP [16]. Consistent with this prior analysis, male infants with placental histology results born to mothers who received IPTp-DP had 23% less malaria than male infants born to mothers who received IPTp-SP (IRR 0.77, 95% CI 0.61-0.99, p=0.049), but this association was not observed in female infants (Table 4.5). Mediation analysis showed that among all infants, 43% of IPTp-DP's effect on preventing malaria during infancy than IPTp-SP was attributed to its greater effect on preventing PM ($IRR_{mediated}$ 0.95, versus IRR_{total} 0.89, Table 4.5). In males, this proportion was 89.7% ($IRR_{mediated}$ 0.79 versus IRR_{total} 0.77). Among female infants, the proportion of mediated effect was not calculated because the direct and mediated effects were in opposing directions.

Discussion

In this secondary analysis of data from a birth cohort of infants born to women randomised to receive monthly IPTp with SP vs DP, infants born to mothers with active PM and severe past PM had a non-significant higher incidence of malaria and complicated malaria during the first year of life compared to infants born to mothers without PM. However, the association between severe past PM and infant malaria was sex-specific. In male, but not female, infants, severe past PM was associated with a significantly higher incidence of malaria, a higher rate of first malaria episode, a higher incidence of complicated malaria, and a higher prevalence of parasitaemia during routine visits, compared to no PM. No sex-specific differences were observed between active PM and the incidence of malaria in infancy. Importantly, male infants born to mothers given IPTp-DP had significantly less malaria in infancy than males born to mothers given IPTp-SP, and 89.7% of this effect was mediated through prevention of PM.

Several prior studies have reported associations between active PM defined as detection of parasites in placental blood or tissue and an increased risk of malaria during infancy [9-11, 23-25]. Active PM detected by microscopy was associated with a higher risk of malaria infection in Ugandan infants [23], an increased rate of first parasitaemia in Beninese infants [11], and a higher risk of first episode of malaria in Gabonese infants [24]. In Mozambique, active PM detected by histology was also associated with higher odds of malaria compared to no PM [9]. The results from the current study, although not statistically significant, are consistent with these prior studies. The exact mechanisms through which PM might impact on the incidence of malaria in infancy are not well understood but may be due to the effects of PM on the foetal immune system, including modulation of innate and adaptive cellular immune responses, as well as altered maternal-foetal transfer of antibodies to *P. falciparum* [26]. Alternatively, these associations may represent confounding secondary to shared levels of exposure to malaria parasites between mothers and their infants. To limit the effect of confounding by malaria exposure in the current study, IPTp arm and markers of exposure, including housing structure and maternal parasitaemia at enrolment were adjusted for, but the possibility of residual confounding persists.

Importantly, in this study, there was an association between the severity of past PM, defined as the proportion of HPF with malaria pigment deposition, with malaria risk, but only in male infants. Severity of malaria pigment deposition in the placenta has been previously reported by our group, as a strong predictor of adverse birth outcomes [15]. To our knowledge, this is the first report to suggest that severity of past PM is also associated with increased risk of malaria in infancy, and that infant sex may modify these associations. Although the precise mechanism by which infant sex modifies the relationship between PM and infant malaria risk remains uncertain, there is a growing body of evidence of sex-based differences in susceptibility to

infectious diseases in infants [27, 28]. While one study in southern Sudan suggested that pregnant women who bore female infants were more likely to have PM than those who bore male infants [29], several adverse pregnancy outcomes, including stillbirths, have been shown to be more common in males than in females [30]. This suggests that *in-utero* fetal exposures may have more severe consequences for male infants than female infants [31]. Furthermore, male infants exposed to malaria *in-utero* have been shown to have higher frequencies of regulatory T cells in cord blood compared to female infants with similar exposure [32], suggesting that *in-utero* malaria exposure may differentially induce tolerance to malaria antigens in male, but not female, infants. Alternatively, other sex-based differences, including malaria-induced responses to toll-like receptor ligands [27, 32], expression of X-chromosome encoded genes [33], and/or glucocorticoid receptor expression [34] may be responsible for these findings. Future studies are needed to better understand how PM may result in different sex-specific outcomes in infants.

IPTp with highly effective drugs such as DP can reduce the severity of PM, and in this study, IPTp-DP was associated with a statistically significant lower incidence of malaria among male infants, and a non-significant lower incidence of malaria among all infants, compared with IPTp-SP. In the mediation analysis, 89.7% of the effect of IPTp-DP vs SP on the incidence of malaria among male infants was mediated through prevention of PM. Although this result was not statistically significant, possibly due to limited sample size to conduct this stratified mediation analysis, these results suggest that in addition to preventing adverse birth outcomes in all infants, effective interventions in pregnancy that reduce severe PM may also result in a lower risk of malaria in male infants.

In this study, there was no significant association between PM and the incidence of non-malaria febrile illnesses in infancy, contrary to other reports that suggest that *in-utero* exposure may also influence immune responses to non-malarial infections [7, 35]. Furthermore, effective prevention of PM with IPTp-DP was not associated with a lower incidence of non-malarial febrile illnesses in infancy compared with IPTp-SP [16]. These data suggest that PM may specifically impact infant malaria risk, although the possibility that it may impact other non-malaria infections cannot be excluded.

This study had some limitations. This secondary analysis was exploratory in nature and did not use categories of PM based on malaria pigment deposition in fibrin used by previous studies [15, 20]. However, prior studies assessed associations between the severity of placental pigment and adverse birth outcomes. To our knowledge, ours is the first study to assess associations between the severity of placental pigment deposition and infant malaria risk. It would be desirable to

come up with a standardized classification system relating the severity of placental pigment deposition to infant malaria risk using data from multiple independent studies from different epidemiological settings. There were relatively small number of infants in the severe past and active PM categories, and our findings should therefore be interpreted with caution. The study was conducted in a very high malaria transmission setting and therefore its findings cannot be generalized to other malaria transmission settings. Finally, exclusion of infants with missing placental histology results (3.2%) and losses to follow-up may have reduced the power of the study and introduced bias if the infants excluded and those lost to follow-up were different from those who completed follow-up. However, there were no significant differences between infants excluded from the analysis, those lost to follow-up, and the infants who completed the study.

Conclusions

Overall, this study suggests that severe malaria pigment deposition in placental tissue is associated with a higher incidence of malaria during the first year of life among infants residing in a setting of high malaria transmission intensity; however, this association was seen in only male infants. Highly effective interventions which reduce both severe past and active PM could be protective in male infants. Future research is needed to evaluate the association between PM and the risk of malaria in infancy in larger studies conducted in moderate and high malaria transmission settings and to evaluate mechanistic pathways between PM and infant malaria risk.

List of abbreviations

aIRR, Adjusted incidence rate ratio

CI, Confidence interval

DP, Dihydroartemisinin-piperaquine

HPF, High-power fields

IOW, Inverse odds weighting

IPTp, Intermittent preventive therapy during pregnancy

IRR, Incidence rate ratio

LAMP, Loop mediated isothermal amplification

PM, Placental malaria

SP, Sulfadoxine-pyrimethamine

Declarations

Ethics approval and consent to participate

The study was approved by the Makerere University School of Biomedical Sciences Ethics Committee, Uganda National Council of Science and Technology, University of California San Francisco Research Ethics Committee, Stanford University Institutional Review Board, and London School of Hygiene and Tropical Medicine Ethics Committee. Parents/guardians of all participants provided written informed consent.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

Funding

This work was supported by grants received from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (grant number: P01 HD059454), the Fogarty International Centers training grant (D43TW7375), the March of Dimes Foundation (Basil O'Connor Award to PJ), and the Bill and Melinda Gates Foundation (OPP1141549). The funders did not play a role in the study design, data collection, analysis, and interpretation, and writing the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

DH, MK, and GD conceived the study with input from AK, PJ, MN, and TC. RK, TO, AK, TC, and GD developed the procedures and wrote the protocol. RK and TO coordinated the fieldwork with input from AK, MN and TR. PJ and HO coordinated the laboratory work. AK conducted the data analysis with support from PJ and MR. SS and DC participated in the analysis, manuscript writing and revision. All authors reviewed the final manuscript and gave permission for publication.

Acknowledgements

We are grateful to the pregnant women and their infants who participated in this study, the administration of Masafu General Hospital, Busia for the support, and staff members of Infectious Diseases Research Collaboration for running the study. This manuscript was part of the first author's PhD studies which were funded by the Fogarty International Center. We are also grateful to Dr Emily Webb for providing statistical advice.

References

1. Sharma L, Shukla G: **Placental malaria: a new insight into the pathophysiology.** *Front Med (Lausanne)* 2017, **4:**117.

2. Walker PG, ter Kuile FO, Garske T, Menendez C, Ghani AC: **Estimated risk of placental infection and low birthweight attributable to *Plasmodium falciparum* malaria in Africa in 2010: a modelling study.** *Lancet Glob Health* 2014, **2:**e460-467.

3. Moore KA, Simpson JA, Scoullar MJL, McGready R, Fowkes FJI: **Quantification of the association between malaria in pregnancy and stillbirth: a systematic review and meta-analysis.** *Lancet Glob Health* 2017, **5:**e1101-e1112.

4. Kapisi J, Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, Ssekitoleko R, Olwoch P, Ategeka J, et al: **Relationships between infection with *Plasmodium falciparum* during pregnancy, measures of placental malaria, and adverse birth outcomes.** *Malar J* 2017, **16:**400.

5. Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, Ruel T, Opira B, Ochokoru H, Ategeka J, Nayebare P, et al: **Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in pregnancy: a double-blind, randomised, controlled, superiority trial.** *Lancet* 2019, **393:**1428-1439.

6. Laufer MK: **Beyond birthweight: benefits and risks of preventing malaria in pregnancy.** *Lancet* 2019, **393:**1388-1390.

7. Dauby N, Goetghebuer T, Kollmann TR, Levy J, Marchant A: **Uninfected but not unaffected: chronic maternal infections during pregnancy, fetal immunity, and susceptibility to postnatal infections.** *Lancet Infect Dis* 2012, **12:**330-340.

8. Park S, Nixon CE, Miller O, Choi NK, Kurtis JD, Friedman JF, Michelow IC: **Impact of malaria in pregnancy on risk of malaria in young children: systematic review and meta-analyses.** *J Infect Dis* 2020.

9. Bardaji A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, Mabunda S, Alonso PL, Menendez C: **Impact of malaria at the end of pregnancy on infant mortality and morbidity.** *J Infect Dis* 2011, **203:**691-699.

10. Boudova S, Divala T, Mungwira R, Mawindo P, Tomoka T, Laufer MK: **Placental but not peripheral *Plasmodium falciparum* infection during pregnancy is associated with increased risk of malaria in infancy.** *J Infect Dis* 2017, **216:**732-735.

11. Le Port A, Watier L, Cottrell G, Ouedraogo S, Dechavanne C, Pierrat C, Rachas A, Bouscaillou J, Bouraima A, Massougbodji A, et al: **Infections in infants during the first 12 months of life: role of placental malaria and environmental factors.** *PLoS ONE [Electronic Resource]* 2011, **6:**e27516.

12. Mutabingwa TK, Bolla MC, Li JL, Domingo GJ, Li X, Fried M, Duffy PE: **Maternal malaria and gravidity interact to modify infant susceptibility to malaria.** *PLoS Med* 2005, **2:**e407.

13. Kakuru A, Staedke SG, Dorsey G, Rogerson S, Chandramohan D: **Impact of *Plasmodium falciparum* malaria and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants: a systematic review.** *Malar J* 2019, **18:**304.

14. Rogerson SJ, Mkundika P, Kanjala MK: **Diagnosis of *Plasmodium falciparum* malaria at delivery: comparison of blood film preparation methods and of blood films with histology.** *J Clin Microbiol* 2003, **41:**1370-1374.

15. Ategeka J, Kakuru A, Kajubi R, Wasswa R, Ochokoru H, Arinaitwe E, Adoke Y, Jagannathan P, R. Kamya M, Muehlenbachs A, et al: **Relationships between measures of malaria at delivery and adverse birth outcomes in a high-transmission area of Uganda.** *The Journal of Infectious Diseases* 2020.

16. Kakuru A, Jagannathan P, Kajubi R, Ochieng T, Ochokoru H, Nakalembe M, Clark TD, Ruel T, Staedke SG, Chandramohan D, et al: **Impact of intermittent preventive treatment of malaria in pregnancy with dihydroartemisinin-piperaquine versus sulfadoxine-pyrimethamine on the incidence of malaria in infancy: a randomized controlled trial.** *BMC Med* 2020, **18:**207.

17. Okiring J, Olwoch P, Kakuru A, Okou J, Ochokoru H, Ochieng TA, Kajubi R, Kamya MR, Dorsey G, Tusting LS: **Household and maternal risk factors for malaria in pregnancy in a highly endemic area of Uganda: a prospective cohort study.** *Malar J* 2019, **18:**144.

18. Hopkins H, Gonzalez IJ, Polley SD, Angutoko P, Ategeka J, Asiimwe C, Agaba B, Kyabayinze DJ, Sutherland CJ, Perkins MD, Bell D: **Highly sensitive detection of malaria parasitemia in a malaria-endemic setting: performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda.** *J Infect Dis* 2013, **208:**645-652.

19. Natureeba P, Ades V, Luwedde F, Mwesigwa J, Plenty A, Okong P, Charlebois ED, Clark TD, Nzarubara B, Havlir DV, et al: **Lopinavir/ritonavir-based antiretroviral treatment (ART) versus efavirenz-based ART for the prevention of malaria among HIV-infected pregnant women.** *J Infect Dis* 2014, **210:**1938-1945.

20. Muehlenbachs A, Fried M, McGready R, Harrington Whitney E, Mutabingwa Theonest K, Nosten F, Duffy Patrick E: **A novel histological grading scheme for placental malaria applied in areas of high and low malaria transmission.** *The Journal of Infectious Diseases* 2010, **202:**1608-1616.

21. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, Hirt R, Cardesa A, Alonso PL: **Placental pathology in malaria: a histological, immunohistochemical, and quantitative study.** *Hum Pathol* 2000, **31:**85-93.

22. Nguyen QC, Osypuk TL, Schmidt NM, Glymour MM, Tchetgen Tchetgen EJ: **Practical guidance for conducting mediation analysis with multiple mediators using inverse odds ratio weighting.** *Am J Epidemiol* 2015, **181:**349-356.

23. De Beaudrap P, Turyakira E, Nabasumba C, Tumwebaze B, Piola P, Boum Ii Y, McGready R: **Timing of malaria in pregnancy and impact on infant growth and morbidity: a cohort study in Uganda.** *Malar J* 2016, **15:**92.

24. Schwarz NG, Adegnika AA, Breitling LP, Gabor J, Agnandji ST, Newman RD, Lell B, Issifou S, Yazdanbakhsh M, Luty AJ, et al: **Placental malaria increases malaria risk in the first 30 months of life.** *Clin Infect Dis* 2008, **47:**1017-1025.

25. Sylvester B, Gasarasi DB, Aboud S, Tarimo D, Massawe S, Mpembeni R, Swedberg G: **Prenatal exposure to *Plasmodium falciparum* increases frequency and shortens time from birth to first clinical malaria episodes during the first two years of life: prospective birth cohort study.** *Malar J* 2016, **15:**379.

26. Harrington WE, Kakuru A, Jagannathan P: **Malaria in pregnancy shapes the development of fetal and infant immunity.** *Parasite Immunol* 2018**:**e12573.

27. Klein SL, Flanagan KL: **Sex differences in immune responses.** *Nature Reviews Immunology* 2016, **16:**626.

28. Muenchhoff M, Goulder PJ: **Sex differences in pediatric infectious diseases.** *J Infect Dis* 2014, **209 Suppl 3:**S120-126.

29. Adam I, Salih MM, Mohmmed AA, Rayis DA, Elbashir MI: **Pregnant women carrying female fetuses are at higher risk of placental malaria infection.** *PLoS One* 2017, **12:**e0182394.

30. Mondal D, Galloway TS, Bailey TC, Mathews F: **Elevated risk of stillbirth in males: systematic review and meta-analysis of more than 30 million births.** *BMC Med* 2014, **12:**220.

31. Goldenberg RL, Andrews WW, Goepfert AR, Faye-Petersen O, Cliver SP, Carlo WA, Hauth JC: **The Alabama Preterm Birth Study: umbilical cord blood *Ureaplasma urealyticum* and *Mycoplasma hominis* cultures in very preterm newborn infants.** *Am J Obstet Gynecol* 2008, **198:**43.e41-45.

32. Prahl M, Jagannathan P, McIntyre TI, Auma A, Wamala S, Nalubega M, Musinguzi K, Naluwu K, Sikyoma E, Budker R, et al: **Sex disparity in cord blood FoxP3(+) CD4 T regulatory cells in infants exposed to malaria in utero.** *Open Forum Infect Dis* 2017, **4:**ofx022.

33. Fish EN: **The X-files in immunity: sex-based differences predispose immune responses.** *Nat Rev Immunol* 2008, **8:**737-744.

34. Clifton VL: **Review: Sex and the human placenta: mediating differential strategies of fetal growth and survival.** *Placenta* 2010, **31 Suppl:**S33-39.

35. Rachas A, Le Port A, Cottrell G, Guerra J, Choudat I, Bouscaillou J, Massougbodji A, Garcia A: **Placental malaria is associated with increased risk of non-malaria infection during the first 18 months of life in a Beninese population.** *Clin Infect Dis* 2012, **55:**672-678.

Figure Legends

Figure 1. Study profile

IPTp=intermittent preventive treatment in pregnancy, DP=dihydroartemisinin-piperaquine, SP=sulfadoxine-pyrimethamine,

Figure 2. Time to first episode of malaria stratified by infant sex

Panel A= all infants, panel B= male infants C=female infants

PM= placental malaria, mild-mod= mild-moderate

No PM=no parasites or pigment detected; active PM=parasites detected with or without pigment

past PM (mild-mod)= >0-20% of high-power fields with pigment but no parasites; past PM(severe)= >20%-60% of high-power fields with pigment but no parasites

Table 4.1 Characteristics of study participants

Characteristic	Maternal IPTp arm	
	Monthly SP (N=327)	Monthly DP (N=329)
Maternal characteristics at enrolment		
Age in years, mean (SD)	24.0 (5.9)	24.0 (5.7)
Gravidity, n (%)		
Primigravida/secundigravida	152 (46.5%)	156 (47.4%)
Multigravida	175 (53.5%)	173 (52.6)
House-hold type, n (%)		
Modern House	77 (23.6%)	71 (21.6%)
Traditional House	250 (76.5%)	258 (78.4%)
Parasite prevalence by microscopy or qPCR, n (%)		
No parasites	53 (16.2%)	63 (19.2%)
Sub-microscopic parasitemia	111 (33.9%)	88 (26.8%)
Microscopic parasitemia	163 (49.9%)	178 (54.1%)
Maternal characteristics during pregnancy		
Parasite prevalence by microscopy, n/N (%)[a]	797/2212 (36.0%)	355/2260 (15.7%)
Incidence of malaria (episodes/ppy)	0.59	0.09
Placental malaria status		
Placental malaria status, n (%)		
No PM	119 (36.4%)	232 (70.5%)
Active PM	71 (21.7%)	7 (2.1%)
Past PM (Mild-moderate pigment)	97 (29.7%)	84 (25.5%)
Past PM (Severe pigment)	40 (12.2%)	6 (1.8%)
Characteristics of infants at birth		
Preterm birth, n (%)	25 (7.7%)	17 (5.2%)
Gestation age in weeks, mean (SD)	39.4 (1.9)	39.6 (1.6)
Low birth weight, n (%)	33 (10.1%)	25 (7.6%)
Birth weight in grams, mean (SD)	3055 (505)	3024 (409)
Female sex, n (%)	161 (49.2%)	175 (53.2%)

Abbreviations: SP, sulfadoxine-pyrimethamine; DP, dihydroartemisinin-piperaquine; SD, standard deviation; PM, placental malaria; ppy, per person year; qPCR, quantitative polymerase chain reaction

[a]Defined as number of routine positive blood smears divided by total number of routine blood smears

No PM=no parasites or pigment detected; active PM=parasites detected with or without pigment

Past PM (Mild-moderate)= >0-20% of high-power fields with pigment but no parasites; Past PM (severe)= >20%-60% of high-power fields with pigment but no parasites

Table 4.2 Association between different measures of placental malaria and incidence of malaria during infancy

Infant category	Placental malaria categories (N)	Malaria episodes	Person years of follow-up	Malaria incidence[a]	Unadjusted		Adjusted[b]	
					IRR (95% CI)	p-value	IRR (95% CI)	p-value
All	No PM (351)	574	327.4	1.75	reference group		reference group	
	Active PM (78)	152	68.9	2.21	1.27 (0.99-1.62)	0.06	1.30 (1.00-1.71)	0.05
	Past PM (mild-mod) (181)	271	157.8	1.72	0.97 (0.79-1.18)	0.74	0.94 (0.76-1.16)	0.55
	Past PM (severe) (46)	91	42.5	2.14	1.22 (0.91-1.63)	0.19	1.28 (0.89-1.83)	0.18
Male	No PM (168)	255	154.5	1.65	reference group		reference group	
	Active PM (45)	86	39.9	2.16	1.31 (0.93-1.87)	0.13	1.27 (0.87-1.84)	0.22
	Past PM (mild-mod) (70)	129	77.7	1.66	0.98 (0.72-1.35)	0.92	1.02 (0.76-1.36)	0.90
	Past PM (severe) (17)	49	15.3	3.20	1.87 (1.29-2.71)	0.001	2.17 (1.45-3.25)	<0.001
Female	No PM (183)	319	172.9	1.84	reference group		reference group	
	Active PM (33)	66	29.0	2.28	1.24 (0.89-1.73)	0.21	1.29 (0.87-1.91)	0.21
	Past PM (mild-mod) (91)	142	80.1	1.77	0.96 (0.73-1.24)	0.74	0.81 (0.61-1.08)	0.15
	Past PM (severe) (29)	42	27.1	1.55	0.85 (0.58-1.24)	0.40	0.74 (0.46-1.20)	0.22

Abbreviations: CI, confidence interval; IRR, incidence rate ratio; mild-mod, mild-moderate; PM, placental malaria

[a] Episodes of malaria per person year of follow-up

[b] Adjusted for gravidity, maternal IPTp, maternal parasitaemia at enrolment, and household type

No PM=no parasites or pigment detected; active PM=parasites detected with or without pigment past PM (mild-mod)= >0-20% of high-power fields with pigment but no parasites; past PM (severe)= >20%-60% of high-power fields with pigment but no parasites

Table 4.3 Association between placental malaria and other malaria outcomes during infancy

Outcome measure	Infant category	Placental malaria categories (N)	Number of cases (incidence PPY)	Unadjusted		Adjusted[a]	
				IRR (95% CI)	p-value	IRR (95% CI)	p-value
Incidence of complicated malaria	All	No PM (351)	30 (0.092)	reference group		reference group	
		Active PM (78)	12 (0.17)	1.89 (0.93-3.87)	0.08	1.72 (0.81-3.66)	0.16
		Past PM (mild-mod) (181)	14 (0.09)	0.97 (0.52-1.81)	0.91	0.99 (0.52-1.92)	0.98
		Past PM (severe) (46)	9 (0.21)	2.30 (0.97-5.43)	0.06	2.44 (0.93-6.37)	0.07
	Male	No PM (168)	13 (0.08)	reference group		reference group	
		Active PM (45)	6 (0.04)	1.78 (0.63-5.02)	0.27	1.18 (0.39-3.62)	0.77
		Past PM (mild-mod) (90)	11 (0.14)	1.68 (0.76-3.68)	0.20	1.54 (0.44-3.45)	0.30
		Past PM (severe) (17)	6 (0.39)	4.61 (1.50-14.22)	0.008	3.88 (1.14-13.03)	0.03
	Female	No PM (183)	17 (0.10)	reference group		reference group	
		Active PM (33)	6 (0.21)	2.09 (0.78-5.64)	0.15	2.60 (0.91-7.44)	0.14
		Past PM (mild-mod) (91)	3 (0.04)	0.38 (0.11-1.29)	0.12	0.44 (0.13-1.54)	0.20
		Past PM (severe) (29)	3 (0.11)	1.12 (0.34-3.66)	0.85	1.63 (0.46-5.81)	0.45
Prevalence measures	**Infant category**	**Placental malaria categories**	**Prevalence n/N (%)**	**Risk ratio (95% CI)**	**p-value**	**Risk ratio (95% CI)**	**p-value**
Prevalence of parasitaemia	All	No PM (351)	377/4170 (9.0%)	reference group		reference group	
		Active PM (78)	82/858 (9.6%)	1.07 (0.76-1.52)	0.70	1.19 (0.82-1.73)	0.36
		Past PM (mild-mod) (181)	154/1993 (7.7%)	0.85 (0.65-1.12)	0.26	0.86 (0.65-1.14)	0.28
		Past PM (severe) (46)	61/542 (11.3%)	1.22 (0.84-1.77)	0.30	1.38 (0.90-2.10)	0.14
	Male	No PM (168)	170/1964 (8.7%)	reference group		reference group	
		Active PM (45)	50/498 (10.0%)	1.17 (0.73-1.86)	0.52	1.19 (0.71-2.00)	0.51
		Past PM (mild-mod) (90)	70/976 (7.2%)	0.81 (0.55-1.20)	0.29	0.87 (0.58-1.31)	0.50
		Past PM (severe) (17)	31/191 (16.2%)	1.80 (1.08-2.99)	0.02	2.24 (1.34-3.74)	0.002
	Female	No PM (183)	207/2206 (9.4%)	reference group		reference group	
		Active PM (33)	32/360 (8.9%)	0.97 (0.57-1.66)	0.91	1.05 (0.61-1.83)	0.85
		Past PM (mild-mod) (91)	84/1017 (8.3%)	0.90 (0.61-1.31)	0.57	0.79 (0.53-1.17)	0.24
		Past PM (severe) (29)	31/351 (8.6%)	0.90 (0.54-1.51)	0.70	0.86 (0.48-1.52)	0.60

Abbreviations: CI, confidence interval; IRR, incidence rate ratio; mild-mod, mild-moderate; PM, placental malaria; PPY, per person year

[a]Adjusted for gravidity, maternal IPTp, maternal parasitaemia at enrolment, and household type

No PM=no parasites or pigment detected; active PM=parasites detected with or without pigment; past PM (mild-mod)= >0-20% of high-power fields with pigment but no parasites; past PM (severe)= >20%-60% of high-power fields with pigment but no parasites

Table 4.4. Association between placental malaria and non-malaria outcomes in infants during the first year of life

Outcome measure	Placental malaria category (N)	Number of cases (incidence PPY)	unadjusted		Adjusted[a]	
			IRR (95% CI)	p-value	IRR (95% CI)	p-value
Incidence of all-cause hospitalisations	No PM (351)	9 (0.027)	reference group		reference group	
	Active PM (78)	3 (0.044)	1.66 (0.40-7.00)	0.49	1.14 (0.22-5.98)	0.87
	Past PM (mild-mod) (181)	7 (0.044)	1.72 (0.58-5.12)	0.33	1.26 (0.44-3.64)	0.66
	Past PM (severe (46)	6 (0.141)	4.85 (1.38-17.00)	0.01	2.90 (0.59-14.44)	0.19
Incidence of non-malarial febrile illnesses	No PM (351)	1128 (3.45)	reference group		reference group	
	Active PM (78)	203 (2.95)	0.86 (0.72-1.03)	0.10	0.84 (0.64-1.10)	0.20
	Past PM (mild-mod) (181)	536 (3.40)	0.98 (0.86-1.13)	0.82	1.03 (0.84-1.25)	0.79
	Past PM (severe (46)	152 (3.58)	1.04 (0.85-1.26)	0.72	1.06 (0.72-1.57)	0.76
Prevalence measures	**Categories**	**Prevalence n/N (%)**	**Risk ratio (95% CI)**	**p-value**	**Risk ratio (95% CI)**	**p-value**
Anaemia[c]	No PM (351)	227/934 (24.3%)	reference group		reference group	
	Active PM (78)	48/190 (25.3%)	1.07 (0.76-1.50)	0.69	1.00 (0.69-1.47)	0.98
	Past PM (mild-mod) (181)	106/441 (24.0%)	1.01 (0.80-1.29)	0.92	0.94 (0.73-1.22)	0.64
	Past PM (severe (46)	37/118 (31.4%)	1.29 (0.94-1.77)	0.12	1.16 (0.79-1.70)	0.44

Abbreviations: CI, confidence interval; IRR, incidence rate ratio; mild-mod, mild-moderate; PM, placental malaria

[a]Adjusted for gravidity, maternal IPTp, maternal parasitaemia at enrolment, and household type

[b] Defined as haemoglobin < 10 g/dL measured routinely at 12, 28, and 52 weeks of age

No PM=no parasites or pigment detected; active PM=parasites detected with or without pigment; past PM (mild-mod)= >0-20% of high-power fields with pigment but no parasites; past PM (severe)= >20%-60% of high-power fields with pigment but no parasites

Table 4.5 Effect of IPTp on malaria incidence in infants that is mediated by preventing placental malaria

Infant category	Total Effect		Direct effect		Mediated effect		% of mediated effect*
	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	
All sexes	0.89 (0.75-1.04)	0.16	0.94 (0.73-1.17)	0.58	0.95 (0.77-1.16)	0.61	43.5%
Males	0.77 (0.61-0.99)	0.05	0.97 (0.67-1.36)	0.89	0.79 (0.56-1.09)	0.17	89.7%
Females	1.01 (0.81-1.26)	0.96	0.92 (0.71-1.22)	0.56	1.09 (0.87-1.38)	0.45	--

Abbreviations: CI, confidence intervals and IRR, incidence rate ratio

Note: IRRs represent the effect of IPTp DP versus SP on the incidence of malaria in infants. Confidence intervals reported here were obtained by bootstrapping and may differ from those reported in the primary analysis which used the delta method specifying robust standard errors.

* Proportion of mediated effect was calculated using $[\ln(IRR_{\text{mediated effect}})/\ln(IRR_{\text{total effect}})]*100$.

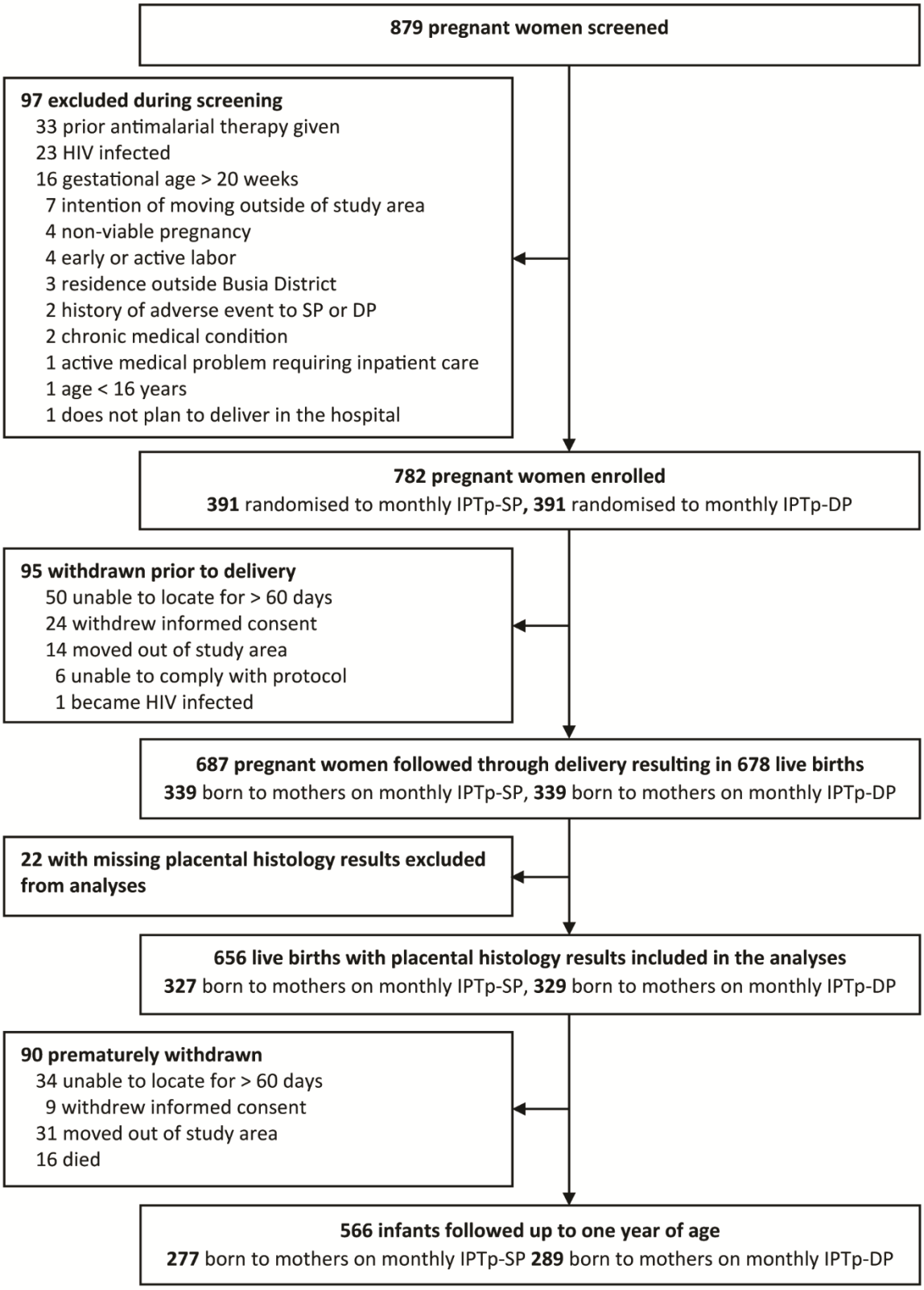

Figure 4.1 Study profile

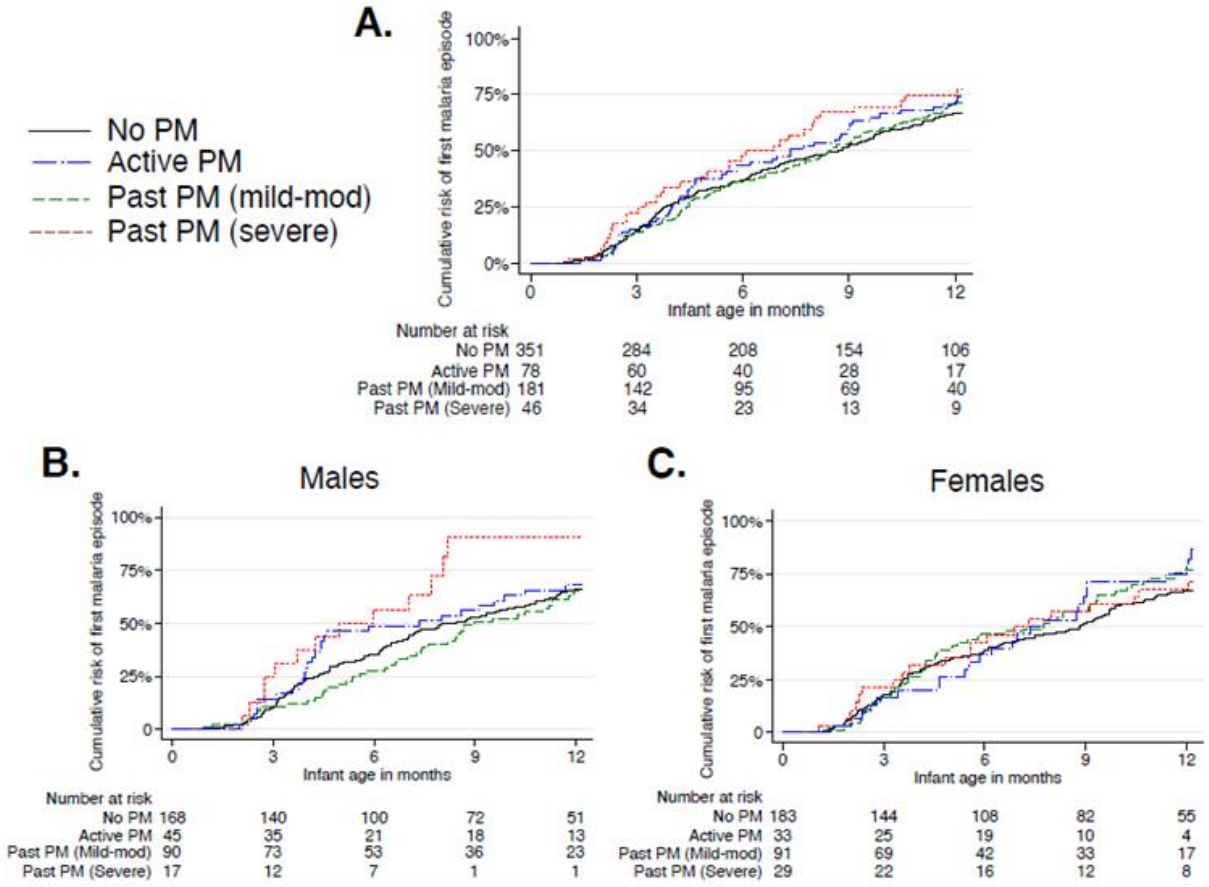

Figure 4.2 Cumulative risk of first malaria episode stratified by placental malaria status

Table 4.6 Additional table of characteristics of study participants stratified by infant sex.

Characteristic	Infant sex	
	Male (N=320)	Female (N=336)
Maternal characteristics at enrolment		
Age in years, mean (SD)	23.9 (5.8)	24.2 (5.9)
Gravidity, n (%)		
Primigravida/secundigravida	152 (47.5%)	156 (46.4%)
Multigravida	168 (52.5%)	180 (53.6%)
House-hold type, n (%)		
Modern House	88 (27.5%)	60 (17.9%)
Traditional House	250 (72.5%)	258 (82.1%)
Parasite prevalence by microscopy or qPCR, n (%)		
No parasites	58 (18.1%)	58 (17.3%)
Sub-microscopic parasitemia	100 (31.3%)	99 (29.5%)
Microscopic parasitemia	162 (50.6%)	179 (53.3%)
Maternal characteristics during pregnancy		
Parasite prevalence by microscopy, n/N (%)[a]	544/2176 (25.0%)	608/2296 (26.5%)
Incidence of malaria (episodes/ppy)	0.33	0.34
Placental malaria status		
Placental malaria status, n (%)		
No PM	168 (52.5%)	183 (54.5%)
Active PM	90 (28.1%)	91 (27.1%)
Past PM (Mild-moderate pigment)	17 (5.3%)	29 (8.6%)
Past PM (Severe pigment)	45 (14.1%)	33 (9.8%)
Characteristics of infants at birth		
Preterm birth, n (%)	17 (5.3%)	25 (7.4%)
Gestation age in weeks, mean (SD)	39.6 (1.5)	39.4 (2.0)
Low birth weight, n (%)	21 (6.6%)	37 (11.0%)
Birth weight in grams, mean (SD)	3108 (438)	2975 (471)

Abbreviations: SP, sulfadoxine-pyrimethamine; DP, dihydroartemisinin-piperaquine; SD, standard deviation; PM, placental malaria; ppy, per person year; qPCR, quantitative polymerase chain reaction

[a]Defined as number of routine positive blood smears divided by total number of routine blood smears

No PM=no parasites or pigment detected; active PM=parasites detected with or without pigment

Past PM (Mild-moderate) = >0-20% of high-power fields with pigment but no parasites; Past PM (severe)= >20%-60% of high-power fields with pigment but no parasites

Characteristics of study participants stratified by infant sex

At enrolment, maternal age was similar between male and female infants. There was no significant difference maternal parasite status at enrolment. The proportion of mothers residing in traditional households was significantly higher in female infants (82.1%) than male infants (72.5%, p=0.003). During pregnancy, maternal parasite prevalence was similar among male and female infants. At birth, the mean birth weight was higher for male infants (mean birth weight=3108, SD=438g) than female infants (mean birth weight= 2975g, SD=471g, p<0.001).

CHAPTER 5 IMPACT OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY ON THE INCIDENCE OF MALARIA DURING INFANCY

5.1 Chapter Introduction

This chapter addresses objective 2, to compare the incidence of malaria during the first year of life in infants born to mothers who were randomised to receive monthly IPTp-DP versus monthly IPTp-SP. The manuscript presents results from a birth cohort of infants born to HIV-uninfected mothers who were randomised to monthly IPTp with DP versus SP, which was published in BMC Medicine in August 2020.

5.2 Research paper

On the next two pages is the publication cover sheet followed by the manuscript, tables, and figures.

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1602857	**Title**	Dr
First Name(s)	Abel		
Surname/Family Name	Kakuru		
Thesis Title	Impact of malaria in pregnancy and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants		
Primary Supervisor	Sarah G. Staedke		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	BMC Medicine		
When was the work published?	August 2020		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	Not applicable		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	

Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I particpated in study conception, participated in obtaining study IRB approvals, coordinated and participated in data collection, conducted data analysis, and wrote the first draft of the manuscript

SECTION E

Student Signature	[redacted]
Date	21/09/2020

Supervisor Signature	[redacted]
Date	21 Sept 2020

Kakuru et al. BMC Medicine (2020) 18:207
https://doi.org/10.1186/s12916-020-01675-x

BMC Medicine

RESEARCH ARTICLE Open Access

Impact of intermittent preventive treatment of malaria in pregnancy with dihydroartemisinin-piperaquine versus sulfadoxine-pyrimethamine on the incidence of malaria in infancy: a randomized controlled trial

Abel Kakuru[1,2*], Prasanna Jagannathan[3], Richard Kajubi[2], Teddy Ochieng[2], Harriet Ochokoru[2], Miriam Nakalembe[4], Tamara D. Clark[5], Theodore Ruel[6], Sarah G. Staedke[1], Daniel Chandramohan[1], Diane V. Havlir[5], Moses R. Kamya[7] and Grant Dorsey[5]

Abstract

Background

Intermittent preventive treatment of malaria during pregnancy (IPTp) with dihydroartemisinin-piperaquine (DP) significantly reduces the burden of malaria during pregnancy compared to sulfadoxine-pyrimethamine (SP), the current standard of care, but its impact on the incidence of malaria during infancy is unknown.

Methods

We conducted a double-blind, randomised trial to compare the incidence of malaria during infancy among infants born to HIV-uninfected pregnant women who were randomised to monthly IPTp with either DP or SP. Infants were followed for all their medical care in a dedicated study clinic and routine assessments were conducted every 4 weeks. At all visits, infants with fever and a positive thick blood smear were diagnosed and treated for malaria. The primary outcome was malaria incidence during the first 12 months of life. All analyses were done by modified intention to treat.

Results

Of the 782 women enrolled, 687 were followed through delivery from December 9, 2016 to December 5, 2017 resulting in 678 live births; 339 born to mothers randomised to SP and 339 born to those randomised to DP. Of these, 581 infants (85.7%) were followed to 12 months of age. Overall, the incidence of malaria was lower among infants born to mothers randomised to DP compared to SP, but the difference was not statistically significant (1.71 vs 1.98 episodes per

person year, incidence rate ratio (IRR) 0.87, 95% confidence interval (CI) 0.73-1.03, p=0.11). Stratifying by infant sex, IPTp with DP was associated with a lower incidence of malaria among male infants (IRR 0.75, 95% CI 0.58-0.98, p=0.03) but not female infants (IRR 0.99, 95% CI 0.79-1.24, p=0.93).

Conclusion

Despite the superiority of DP for IPTp, there was no evidence of a difference in malaria incidence during infancy in infants born to mothers who received DP compared to those born to mothers who received SP. Only male infants appeared to benefit from IPTp-DP suggesting that IPTp-DP may provide additional benefits beyond birth. Further research is needed to further explore the benefits of DP versus SP for IPTp on health outcomes of infants.

Key words

malaria, intermittent preventive treatment, pregnancy, sulfadoxine-pyrimethamine, dihydroartemisinin-piperaquine, infants

* Correspondence: akakuru@idrc-uganda.org
[1]London School of Hygiene and Tropical Medicine, London, UK
[2]Infectious Diseases Research Collaboration, Kampala, Uganda
Full list of author information is available at the end of the article

Background

Infection with malaria parasites during pregnancy remains a major public health problem, especially in sub-Saharan Africa where transmission intensity is highest and *Plasmodium falciparum* the predominant species. In 2018 there were an estimated 39 million pregnancies in sub-Saharan Africa, of which over 11 million (29%) were exposed to *P. falciparum* [1]. The majority of women residing in malaria endemic areas of sub-Saharan Africa are partially immune and do not develop symptoms when infected with *P. falciparum* during pregnancy. However, even in the absence of symptomatic disease, malaria in pregnancy is associated with maternal anaemia and adverse birth outcomes such as low birth weight, preterm delivery, and stillbirth [2-4].

To prevent malaria in pregnancy and improve birth outcomes, the World Health Organization (WHO) recommends intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) in pregnant women residing in areas of moderate to high malaria transmission intensity [5]. However, the effectiveness of IPTp-SP is threatened by widespread antifolate resistance resulting in failure to clear parasites and prevent new infections [6]. Recent studies have shown dihydroartemisinin-piperaquine (DP) to be a promising alternative to SP for IPTp. Compared to IPTp-SP, IPTp-DP has been shown to be much more effective at reducing the prevalence of malaria parasitaemia and incidence of clinical malaria during pregnancy and reducing the risk of placental malaria at delivery [7-9]. However, despite significantly reducing the burden of malaria during pregnancy, IPTp-DP has not been shown to be superior to IPTp-SP at improving adverse birth outcomes [7-9].

Prevention of malaria during pregnancy may have additional benefits to the infant beyond the neonatal period. Studies have shown that intrauterine exposure to *P. falciparum* may negatively affect the development of antimalarial immunity in the infant [10, 11]. Indeed, several observational studies have suggested that placental malaria increases the risk of malaria during infancy [12-14]. However, these studies could not rule out the possible confounding effect of behavioural, environmental, genetic, and social-economic factors shared by the mother and her infant on the associations between exposure to malaria parasites during pregnancy and risk of malaria during infancy. A more robust method of testing this hypothesis would be to compare the impact of a highly effective regimen versus a less effective regimen for IPTp on the risk of malaria during infancy in a randomised controlled trial. However, to date clinical trials that have evaluated the impact of IPTp on the risk of malaria during infancy have been limited by similar efficacy of IPTp regimens [15, 16] or the provision of chemoprevention during infancy, in addition to IPTp [17].

To address this gap in evidence, we compared the incidence of malaria during the first year of life among infants born to HIV-uninfected pregnant women who took part in a double-blind, randomised, controlled trial of monthly IPTp with DP (a highly effective regimen) versus SP (a less effective regimen). During pregnancy, IPTp-DP was superior to IPTp-SP for reducing the incidence of clinical malaria and prevalence of asymptomatic parasitaemia during pregnancy, and the prevalence of placental malaria at delivery [9]. Children born to mothers enrolled in this study did not receive chemoprevention during infancy.

Methods

Study setting and participants

This study was conducted from September 6, 2016 to December 4, 2018, in Busia district, south-eastern Uganda, an area of high malaria transmission intensity. The study was conducted in two phases; the pregnancy phase, which involved enrolment and follow-up of pregnant women through delivery, and the infancy phase which involved follow-up of infants through 12 months of age. Details of the pregnancy phase of the study have been published elsewhere [9]. In brief, HIV-uninfected pregnant women were eligible for enrolment if they were 12-20 weeks of gestation, 16 years or older, agreed to come to the study clinic for any illness, had no history of taking IPTp-SP or any other antimalarial therapy during the current pregnancy, and provided written informed consent. Women were excluded if they had a history of serious adverse events to SP or DP. The infancy phase involved the follow-up of all live births among women enrolled in the pregnancy phase of the study.

Study design, randomisation, and masking

This was a double-blind, randomised, controlled trial designed to assess the impact of monthly IPTp with DP versus SP in HIV-uninfected pregnant women, on the incidence of malaria during infancy (Trial registration, ClinicalTrials.gov; NCT02793622). At enrolment, pregnant women were randomly assigned in a 1:1 ratio to receive IPTp-DP or IPTp-SP. A randomisation list was computer generated using permuted blocks of 4 or 8 by a staff member not directly involved in patient care. To achieve allocation concealment, sealed envelopes, each containing a treatment allocation number and treatment group assignment, were prepared following the sequence of the randomisation list, prior to enrolment. Treatment allocation was done by a study pharmacist not involved in daily patient care by picking the next available sealed envelope, recording the participant's identification number on the envelope, and opening it to reveal the assigned treatment. The treatment allocation number, the participant's identification number, and the assigned treatment were then recorded on a treatment allocation log which was kept in a safe

lockable place only accessible by the study pharmacist. Study drugs for each enrolled participant were pre-packaged by the study pharmacist and labelled with the participant's identification number. Study drugs were administered every 4 weeks starting at 16 or 20 weeks of gestation. Each dose of DP (tablets of 40 mg of dihydroartemisinin and 320 mg of piperaquine; Duo-Cotexin, Holley-Cotec, Beijing, China) consisted of 3 tablets given once a day for 3 consecutive days. Each dose of SP (tablets of 500 mg of sulfadoxine and 25 mg of pyrimethamine; Kamsidar, Kampala Pharmaceutical Industries) consisted of 3 tablets given as a single dose. To achieve blinding, participants randomised to DP also received SP placebos, and participants randomised to SP received DP placebos every 4 weeks. All 1st daily doses of study drugs were administered directly observed in the clinic. The 2nd and 3rd daily doses were dispensed to the mother for self-administration at home. Adherence to the 2nd and 3rd daily doses was assessed by self-reporting during the visits following routine visits.

Study procedures

Study procedures for pregnant women have been previously described in detail [9]. Briefly, at enrolment, all participants received a long-lasting insecticide treated net and underwent a standard history and physical examination. Pregnant women were encouraged to come to a dedicated study clinic any time they were ill and to attend routine visits conducted every 4 weeks for study drug dispensing and laboratory testing. Pregnant women diagnosed with symptomatic malaria detected by microscopy at any visit were treated with artemether-lumefantrine. Asymptomatic parasitaemia detected during routine visits was not treated. At delivery, a standardised assessment was completed including evaluation of birth weight, gestation age based on ultrasound dating, and collection of biological specimens including placental tissue and placental blood.

Following delivery, all live births were followed up to 12 months of age. Mothers were encouraged to bring their infants to a dedicated study clinic open every day for all their medical care and were provided transport refund. Routine assessments were conducted every 4 weeks including collection of blood for the detection of parasites by microscopy. Infants who presented with a history of fever in the past 24 hours or with a documented tympanic temperature $\geq$ 38.0˚C had blood collected for a thick blood smear. If the thick blood smear was positive, infants were treated for malaria according to the Uganda Ministry of Health guidelines which consisted of artemether-lumefantrine for uncomplicated malaria and intravenous artesunate for complicated malaria. Non-malarial illnesses were treated according to the integrated management of childhood illnesses guidelines. At 12, 28, and 52 weeks of age, blood was collected for haemoglobin measurement.

Laboratory procedures

Presence of malaria parasites in dried placental blood spots was detected by loop-mediated isothermal amplification as previously described [18]. Placental malaria by histology defined as presence of malaria parasites or malaria pigment was detected from placental tissue as previously described [19]. Blood smears were stained with 2% Giemsa and read by experienced microscopists. A blood smear was considered negative when the examination of 100 high power fields did not reveal asexual parasites. All blood smears were read by two independent microscopists. Blood smears with discrepant results between the first and second reader were read by a third reader as a tie breaker. Haemoglobin measurements were made using a portable spectrophotometer (Hemocue, Angelholm, Sweden).

Study outcomes

The primary outcome was the incidence of malaria from birth to 12 months of age. An incident episode of malaria was defined as the presence of fever (history of fever in the past 24 hours or a tympanic temperature ≥ 38.0˚C) with a positive thick blood smear not preceded by another malaria episode in the last 14 days. Secondary outcomes included time to a first episode of malaria; incidence of complicated malaria defined as an episode of malaria with danger signs (any of the following, less than 3 convulsions over 24 hours, inability to sit or stand, vomiting everything, unable to breastfeed or drink) or meeting standardised criteria for severe malaria; incidence of all cause hospitalisations; infant mortality; incidence of non-malarial febrile illnesses; prevalence of malaria parasitaemia during routine visits; and prevalence of anaemia (haemoglobin <10g/dL) at 12, 28, and 52 weeks of age.

Statistical analysis

To test the hypothesis that infants born to mothers randomised to IPTp-DP would have a lower incidence of malaria during the first 12 months of life compared to infants born to mothers randomised to IPTp-SP, it was estimated that the incidence of malaria in infants born to mothers randomised to IPTp-SP would be 3-5 episodes per person year (using data from a prior study conducted in the adjacent district of Tororo [20] and a loss of 5% of follow-up time per year). With these assumptions, the study had 80% power to detect an 18-23% difference in the incidence of malaria (incidence rate ratio of 0.77-0.82) among infants born to mothers randomised to IPTp-DP compared to those born to mothers randomised to IPTp-SP with a two-sided significance level of 0.05.

Data were double entered and verified in Microsoft Access by two independent data entrants. Using Stata, version 14.2, statistical analyses were done in the modified intention-to-treat

population, which included all live births followed until they reached 12 months of age or premature study withdrawal. Comparisons of simple proportions were made using the chi-square test or Fisher's exact test. Comparisons of continuous variables were made using the t-test. Comparisons of proportions with repeated measures were made with generalised estimating equations, with the use of log-binomial regression and robust standard errors to adjust for clustering. Comparisons of incidence measures were made using a negative binomial regression model. Incidence rate ratios (IRRs) were defined as the incidence in the IPTp-DP arm divided by the incidence in the IPTp-SP arm. Prevalence ratios were defined as the prevalence in the IPTp-DP arm divided by the prevalence in the IPTp-SP arm. Stratified analyses of our primary outcome according to infant sex and age, and maternal gravidity were planned a priori in the statistical analysis plan. The cumulative risk of the first episode of malaria was compared using a Cox proportional hazards model with the association expressed as the hazard ratio (HR). In all analyses, two-sided p-values of <0.05 were considered statistically significant.

Results

Study participants and follow-up

Between September 2016 and May 2017, 879 pregnant women were screened and 782 were enrolled and randomised to receive either monthly IPTp with DP (N=391) or SP (N=391) (figure 1) [9]. Among the 687 (87.9%) women who were followed through delivery, there were 678 live births from December 9, 2016 to December 5, 2017, 339 in the IPTp-DP group and 339 in the IPTp-SP group. Among the 678 live births, 581 infants (85.7%) were followed up to 12 months of age (Figure 5.1). Maternal characteristics at enrolment were similar between the two groups (Table 5.1). Mean age at enrolment was 23.9 years, 321 (47.3%) infants were born to primigravida or secundigravida mothers, and 399 (58.8%) infants were born to mothers enrolled at 12-16 weeks of gestation while 279 (41.2%) were born to mothers enrolled at >16-20 weeks of gestation. Three hundred fifty-three (52.1%) infants were born to mothers with parasitaemia detected by microcopy at enrolment. During pregnancy, the period prevalence of parasitaemia detected by microscopy during routine visits was significantly lower in mothers who received IPTp-DP (365/2324, 15.7%) compared to mothers who received IPTp-SP (828/2291, 36.1%, p<0.001). Similarly, the incidence of malaria during pregnancy was significantly lower in mothers who received IPTp-DP compared to mothers who received IPTp-SP (0.09 vs 0.59 episodes per person year, p<0.001). At delivery, compared to mothers who received IPTp-SP, mothers who received IPTp-DP had a significantly lower prevalence of placental parasitaemia detected by histology (28.3% vs 60.9%, p<0.001, Table 5.1). However, at birth, there were no significant

differences in characteristics of infants born to mothers who received IPTp-DP or IPTp-SP (Table 5.1).

Impact of IPTp with DP on incidence of malaria in infancy

Overall, infants born into the cohort experienced 1131 episodes of malaria during 614 person years of follow-up. The incidence of malaria was lower among infants born to mothers who received IPTp-DP (1.71 episodes per person year) compared to infants born to mothers who received IPTp-SP (1.98 episodes per person year), but the difference was not statistically significant (incidence rate ratio (IRR) 0.87, 95% confidence interval (CI) 0.73-1.03, p=0.11; Table 5.2). However, the association between IPTp and the incidence of malaria in infants was modified by infant sex. Among male infants, the incidence of malaria was significantly lower among infants born to mothers who received IPTp-DP compared to those born to mothers who received IPTp-SP (IRR 0.75, 95% CI 0.58-0.98, p=0.03). There was no difference in the incidence of malaria between female infants born to mothers who received IPTp-DP versus IPTp-SP (IRR 0.99, 95% CI 0.79-1.24, p=0.93; Table 5.2). In addition, difference in malaria incidence between the IPTp regimens among male infants was only significant between >3 to 12 months of age (IRR 0.73, 95% CI 0.56-0.96, p=0.02) but not between 0 to 3 months of age (IRR 0.89, 95% CI 0.48-1.66, p=0.72). There were no differences between the IPTp regimens among female infants stratified by age (table 5.2). There was no significant difference in the time to first episode of malaria between infants born to mothers who received IPTp-DP and infants born to mothers who received IPTp-SP (hazard ratio (HR) 0.90, 95% CI 0.75-1.09, p=0.30). When stratified by infant sex, male infants born to mothers who received IPTp-DP had a lower rate of first episode of malaria compared to male infants born to mothers who received IPTp-SP but this did not reach statistical significance (HR 0.79, 95% CI 0.60-1.05, p=0.10) and there was no difference among female infants (HR 1.01, 95% CI 0.78-1.31, p=0.96; Figure 5.2).

Impact of IPTp with DP on incidence of complicated malaria in infancy

Overall, there were 68 episodes of complicated malaria among 59 different infants. For 60 episodes of complicated malaria, only danger signs were present while 8 episodes met criteria for severe malaria (7 episodes of respiratory distress and 1 episode of severe anaemia). The incidence of complicated malaria was lower among infants born to mothers who received IPTp-DP compared to those born to mothers who received IPTp-SP (IRR=0.54, 95% CI 0.32-0.92, p=0.02; Table 5.3). Again, effect modification by infant sex was observed. Male infants born to mothers who received IPTp-DP had a significantly lower incidence of complicated malaria compared to male infants born to mothers who received IPTp-SP (IRR 0.36, 95% CI 0.17-0.78, p=0.01). There was no significant difference in the incidence of complicated malaria between

female infants born to mothers who received IPTp-DP or IPTp-SP (IRR 0.86, 95% CI 0.40-1.87, p=0.71).

Impact of IPTp with DP on other secondary outcomes

The incidence of all cause hospitalisations was lower among infants born to mothers who received IPTp-DP compared to infants born to mothers who received IPTp- SP, but this did not reach statistical significance (IRR 0.39, 95% CI 0.15-1.05, p=0.06). When stratified by sex, male infants born to mothers who received IPTp-DP had a statistically significantly lower incidence of all cause hospitalisations compared to male infants born to mothers who received IPTp-SP (IRR 0.20, 95% CI 0.05-0.82, p=0.03), but there was no difference among female infants (IRR 1.01, 95% CI 0.22-4.63, p=0.99). A total of 16 infants died, including 9 in the neonatal period. One infant born to a mother who received IPTp-DP died of malaria at 11 months of age. There was no significant difference in the mortality rate between infants born to mothers who received IPTp-DP or IPTp-SP (IRR 0.45, 95% CI 0.03-7.88, p=0.59). The incidence of non-malarial febrile illnesses was similar among infants born to mothers who received IPTp-DP or IPTp-SP. The prevalence of parasitaemia during routine visits and prevalence of anaemia (haemoglobin<10g/dL) measured at 12, 28, and 52 weeks of age were similar among infants born to mothers who received IPTp-DP or IPTp-SP. Considering time to first parasitaemia, there was no significant difference between infants born to mothers who received IPTp-DP and those born to mothers who received IPTp-SP (HR 0.92, 95% CI 0.76-1.10, p=0.37). When stratified by infant sex, male infants born to mothers who received IPTp-DP had a slightly lower rate of first parasitaemia compared to male infants born to mothers who received IPTp-SP but this did not reach statistical significance (HR 0.81, 95% CI 0.62-1.06, p=0.13) and there was no difference among female infants (HR 1.02, 95% CI 0.79-1.31, p=0.87).

Discussion

In this double-blind, randomised, controlled trial conducted in an area of high malaria transmission intensity, infants born to mothers randomised to IPTp-DP had a 13% lower incidence of malaria compared to infants born to mothers randomised to IPTp-SP, but the difference was not statistically significant. However, among male infants, IPTp-DP was associated with a lower incidence of malaria compared to IPTp-SP. This difference was primarily seen between >3-12 months of age. Similarly, male infants born to mothers who received IPTp-DP had a lower incidence of complicated malaria and all cause hospitalisations compared to male infants born to mothers who received IPTp-SP. In contrast, among female infants there

were no differences in the incidence of malaria, complicated malaria, and all cause hospitalisations between those born to mothers who received IPTp-DP versus IPTp-SP. For both sexes, there were no significant associations between maternal IPTp regimen and the risk of non-malarial febrile illness or anaemia during infancy.

IPTp-DP remains an attractive alternative to IPTp-SP for the prevention of malaria in HIV-uninfected pregnant women. Three randomised controlled trials conducted recently in East Africa have consistently shown that IPTp-DP is associated with significant reductions in the risk of maternal parasitaemia, maternal clinical malaria and placental malaria compared to IPTp-SP, yet, none of these studies has shown significant differences in the risk of adverse birth outcomes for infants born to mothers receiving DP or SP for IPTp [7-9]. Historically, IPTp policy recommendations have been driven by evidence supporting reductions in the risk of adverse birth outcomes, specifically low birth weight, and for this reason the WHO continues to recommend IPTp-SP [5]. However, improved protection from malaria infection during pregnancy may have longer term effects on infant health [21]. A wealth of immunologic evidence suggests that maternal malaria infection affects the development of the foetal and infant immune system, both by altering the immune graft received by the foetus (both maternal antibodies and cells) as well as exposing the foetus to parasite antigens that may alter both the innate and adaptive immune responses [22]. Indeed, infants born to mothers with placental malaria may be at an increased risk of malaria during infancy and it would be important to know if more effective IPTp regimens could reduce the risk of malaria in infants [13, 14].

In this study, although there was no overall evidence of a reduced malaria incidence in infants born to mothers receiving IPTp-DP versus IPTp-SP, our overall findings suggest that IPTp-DP may be associated with a moderately lower incidence of malaria in infants. A recently published systematic review identified three randomised controlled trials which evaluated the impact of different IPTp regimens on malaria during infancy [23]. There were no significant differences in the incidence of malaria among infants born to mothers randomised to IPTp with mefloquine versus IPTp-SP [16], intermittent screening and treatment with AL versus IPTp-SP [15], or IPTp-SP versus placebo [14]. However, these studies were limited by the failure of the alternative (non-SP) regimen to significantly reduce the risk of placental malaria. In a randomised controlled trial from Uganda, IPTp-DP was found to significantly reduce the risk of placental malaria compared to IPTp-SP [8]. But, surprisingly, the incidence of malaria during the first two years of life in infants born into this cohort was higher in infants born to mothers who received monthly IPTp-DP compared to those born to mothers who received IPTp-SP given every two months [17]. However, in that study all infants were also given DP every 3 months, and the association between IPTp-DP and increased malaria during infancy was only observed in females. This

finding could largely be explained by the fact that female infants with *in utero* exposure to DP had lower piperaquine levels after receiving DP during infancy, which is strongly predictive of malaria risk [24]. In contrast, among male infants, *in utero* exposure to DP was not associated with piperaquine levels during infancy [17]. Furthermore, male infants born to mothers randomised to IPTp-DP had a trend towards a lower incidence of malaria compared to male infants born to mothers who received IPTp-SP, a similar finding to that reported in here (aIRR 0.66, 95% CI 0.25-1.75) [17].

In the present study, IPTp-DP was associated with a lower incidence of malaria, a lower incidence of complicated malaria and a lower incidence of all cause hospitalisations during infancy compared to IPTp-SP, but only among male infants. Although the precise mechanism by which infant sex modifies the relationship between maternal IPTp, maternal malaria infection and infant malaria risk remains uncertain, there is a growing body of evidence of sex-based differences in susceptibility to infectious diseases in infants [25, 26]. Male infants have been found to have an increased pre-disposition to more frequent and more severe manifestations of infectious diseases than females [26]. Similarly, several adverse pregnancy outcomes, including stillbirth, are more common in males than in females [27]. This suggests that *in utero* foetal exposures may have more severe consequences for male infants than female infants [28]. Several potential mechanisms for these sex-based differences have been described. These include genetic differences attributable to the heterogeneity of expression of X-chromosome encoded genes [29], sex-dependent differences in glucocorticoid receptor expression and foetal-placental responsivity to cortisol [30], and sex-specific differences in neonatal and infant immune responses to toll-like receptor ligands and induction of regulatory T cell populations [25, 31]. Given the reduced risk of malaria among male infants whose mothers received IPTp-DP compared to those whose mothers received IPTp-SP, we hypothesise that effective prevention of maternal malaria may prevent sex-specific adverse consequences of placental malaria.

Our study had some limitations. The observed incidence of malaria in infants born to mothers who received IPTp-SP (1.98 episodes per person year) was lower than the assumed incidence of 3-5 episodes per person per year which was used for sample size estimation. This limited the power of the study to detect a significant difference between the two IPTp arms if such a difference truly existed. Also, our study was not powered to conduct stratified analyses by infant sex and age, or to test for associations between IPTp-DP and secondary outcomes such as complicated malaria and all cause hospitalizations, which were relatively uncommon in our study. Therefore, statistically significant associations observed in stratified analyses and between IPTp-DP and secondary outcomes should be interpreted with caution. This study was conducted in an area with very high malaria transmission intensity, as at enrolment over 80% of

mothers had malaria parasitaemia detected by microscopy or quantitative PCR [9]. This limits the generalisation of our findings to other areas with lower transmission intensity. Also, administration of all SP doses was directly observed while only the 1st daily dose of DP was directly observed. It is possible that some mothers did not take the 2nd and 3rd dose of DP which were dispensed for self-administration at home. This could have led to underestimation of the effect of IPTp-DP compared to SP on the incidence of malaria. Finally, we measured malaria incidence using passive surveillance which could have underestimated the true incidence of malaria if infants were treated for malaria at home or taken for care outside the study clinic. However, mothers were encouraged to bring their infants to the study clinic for care whenever they were sick and were provided transport refund.

Conclusion

In summary, our study findings show that in addition to reducing the burden of malaria during pregnancy and placental malaria at delivery compared to monthly IPTp-SP [9], monthly IPTp-DP was associated with a reduced incidence of malaria, complicated malaria and a trend towards reduced incidence of all cause hospitalisations among male infants born to HIV-uninfected pregnant women residing in a high malaria transmission setting. Improved prevention of malaria during pregnancy may have additional benefits beyond birth. These results provide additional support for replacing SP with DP for IPTp in areas with widespread antifolate resistance. Future studies of IPTp should consider follow-up of infants beyond the neonatal period to evaluate the potential impact of IPTp on infant outcomes, stratify results based on infant sex, and evaluate the impact of IPTp on infant outcomes in settings of moderate malaria transmission intensity.

List of abbreviations

CI: confidence interval

DP: dihydroartemisinin-piperaquine

HR: hazard ratio

IPTp: intermittent preventive treatment of malaria in pregnancy

IRR: incident rate ratio

LAMP: loop-mediated isothermal amplification

PR: prevalence ratio

SP: sulfadoxine-pyrimethamine

Declarations

Ethical approval and consent to participate

The study was approved by Makerere University School of Biomedical Sciences Ethics Committee (approval number SBS-342), the Uganda National Council of Science and Technology (HS 2052), and the University of California San Francisco Research Ethics Committee (approval number16-18679). In addition, retrospective approval for the infancy phase of the study was obtained from the London School of Hygiene and Tropical Medicine Ethics Committee. The parents/guardians of all participants provided written informed consent before enrolment.

Consent for publication

Not applicable

Availability of data and materials

Data collected for the study including individual participant data and data dictionaries defining fields in the datasets have been made available to others through request to the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Data and Specimen Hub (DASH): https://dash.nichd.nih.gov/Resource/Tutorial. Data can be accessed through the NICHD-DASH website: https://dash.nichd.nih.gov/Study/20027 following user registration and a research data request process. The NICHD DASH Data Access Committee reviews all requests to determine that a requester's proposed use of the data is scientifically and ethically appropriate and does not conflict with constraints or informed consent limitations identified by the institutions that submitted the data.

Competing interests

All authors declare that they have no competing interests.

Funding

This study was funded through grants received from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (P01 HD059454), and the Bill and Melinda Gates Foundation (OPP1141549). The funders did not play a role in the study design; data

collection, analyses, and interpretation; manuscript preparation; and in the decision to submit the manuscript for publication.

Authors' contributions

DH, MK, and GD conceived of the study with input from AK, PJ, MN, and TC. RK, TO, AK, TC, and GD developed the procedures and wrote the protocol. RK and TO coordinated the fieldwork with input from AK, MN and TR. PJ and HO coordinated the laboratory work. AK conducted the data analysis with support from GD and PJ. SS and DC participated in the analysis, manuscript writing and revision. All authors read and approved the final manuscript.

Acknowledgements

We thank the pregnant women and their infants who participated in the study, the administration, and practitioners of Masafu General Hospital Busia and staff members of Infectious Diseases Research Collaboration. This manuscript was part of the first author's PhD studies which were funded by the Fogarty International Center.

References

1. **World malaria report 2019** [https://www.who.int/publications-detail/world-malaria-report-2019]. Accessed 21 Mar 2020.

2. Walker PG, ter Kuile FO, Garske T, Menendez C, Ghani AC: **Estimated risk of placental infection and low birthweight attributable to *Plasmodium falciparum* malaria in Africa in 2010: a modelling study**. *The Lancet Global health* 2014, **2**(8):e460-467.

3. Kapisi J, Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, Ssekitoleko R, Olwoch P, Ategeka J *et al*: **Relationships between infection with *Plasmodium falciparum* during pregnancy, measures of placental malaria, and adverse birth outcomes**. *Malar J* 2017, **16**(1):400.

4. Moore KA, Simpson JA, Scoullar MJL, McGready R, Fowkes FJI: **Quantification of the association between malaria in pregnancy and stillbirth: a systematic review and meta-analysis**. *The Lancet Global health* 2017, **5**(11):e1101-e1112.

5. **WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP).** [http://www.who.int/malaria/publications/atoz/iptp-sp-updated-policy-brief-24jan2014.pdf]

6. Desai M, Gutman J, Taylor SM, Wiegand RE, Khairallah C, Kayentao K, Ouma P, Coulibaly SO, Kalilani L, Mace KE *et al*: **Impact of sulfadoxine-pyrimethamine resistance on effectiveness of intermittent preventive therapy for malaria in pregnancy at clearing infections and preventing low birth weight**. *Clin Infect Dis* 2016, **62**(3):323-333.

7. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, Ouma P, Were V, Laserson K, Katana A *et al*: **Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial**. *Lancet* 2015, **386**(10012):2507-2519.

8. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, Opira B, Olwoch P, Ategeka J, Nayebare P *et al*: **Dihydroartemisinin-piperaquine for the prevention of malaria in pregnancy**. *N Engl J Med* 2016, **374**(10):928-939.

9. Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, Ruel T, Opira B, Ochokoru H, Ategeka J, Nayebare P *et al*: **Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in pregnancy: a double-blind, randomised, controlled, superiority trial**. *Lancet* 2019, **393**(10179):1428-1439.

10. Ismaili J, Van Der Sande M, Holland MJ, Sambou I, Keita S, Allsopp C, Ota MO, McAdam KPWJ, Pinder M: ***Plasmodium falciparum* infection of the placenta affects newborn immune responses**. *Clin Exp Immunol* 2003, **133**(3):414-421.

11. Brustoski K, Moller U, Kramer M, Hartgers FC, Kremsner PG, Krzych U, Luty AJ: **Reduced cord blood immune effector-cell responsiveness mediated by CD4+ cells induced in utero as a consequence of placental *Plasmodium falciparum* infection**. *J Infect Dis* 2006, **193**(1):146-154.

12. Boudova S, Divala T, Mungwira R, Mawindo P, Tomoka T, Laufer MK: **Placental but not peripheral *Plasmodium falciparum* infection during pregnancy is associated with increased risk of malaria in infancy**. *J Infect Dis* 2017, **216**(6):732-735.

13. Le Port A, Watier L, Cottrell G, Ouedraogo S, Dechavanne C, Pierrat C, Rachas A, Bouscaillou J, Bouraima A, Massougbodji A *et al*: **Infections in infants during the first 12 months of life: role of placental malaria and environmental factors**. *PLoS ONE [Electronic Resource]* 2011, **6**(11):e27516.

14. Bardaji A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, Mabunda S, Alonso PL, Menendez C: **Impact of malaria at the end of pregnancy on infant mortality and morbidity**. *J Infect Dis* 2011, **203**(5):691-699.

15. Awine T, Belko MM, Oduro AR, Oyakhirome S, Tagbor H, Chandramohan D, Milligan P, Cairns M, Greenwood B, Williams JE: **The risk of malaria in Ghanaian infants born to women managed in pregnancy with intermittent screening and treatment for malaria or intermittent preventive treatment with sulfadoxine/pyrimethamine**. *Malar J* 2016, **15**:46.

16. Ruperez M, Gonzalez R, Mombo-Ngoma G, Kabanywanyi AM, Sevene E, Ouedraogo S, Kakolwa MA, Vala A, Accrombessi M, Briand V *et al*: **Mortality, morbidity, and developmental outcomes in infants born to women who received either mefloquine or sulfadoxine-pyrimethamine as intermittent preventive treatment of malaria in pregnancy: a cohort study**. *PLoS Medicine / Public Library of Science* 2016, **13**(2):e1001964.

17. Jagannathan P, Kakuru A, Okiring J, Muhindo MK, Natureeba P, Nakalembe M, Opira B, Olwoch P, Nankya F, Ssewanyana I *et al*: **Dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria during pregnancy and risk of malaria in early childhood: a randomized controlled trial**. *PLoS Med* 2018, **15**(7):e1002606.

18. Hopkins H, Gonzalez IJ, Polley SD, Angutoko P, Ategeka J, Asiimwe C, Agaba B, Kyabayinze DJ, Sutherland CJ, Perkins MD *et al*: **Highly sensitive detection of malaria parasitemia in a malaria-endemic setting: performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda**. *J Infect Dis* 2013, **208**(4):645-652.

19. Natureeba P, Ades V, Luwedde F, Mwesigwa J, Plenty A, Okong P, Charlebois ED, Clark TD, Nzarubara B, Havlir DV *et al*: **Lopinavir/ritonavir-based antiretroviral treatment (ART) versus efavirenz-based ART for the prevention of malaria among HIV-infected pregnant women**. *J Infect Dis* 2014, **210**(12):1938-1945.

20. Jagannathan P, Muhindo MK, Kakuru A, Arinaitwe E, Greenhouse B, Tappero J, Rosenthal PJ, Kaharuza F, Kamya MR, Dorsey G: **Increasing incidence of malaria in children despite insecticide-treated bed nets and prompt anti-malarial therapy in Tororo, Uganda**. *Malar J* 2012, **11**:435.

21. Dauby N, Goetghebuer T, Kollmann TR, Levy J, Marchant A: **Uninfected but not unaffected: chronic maternal infections during pregnancy, fetal immunity, and susceptibility to postnatal infections**. *Lancet Infect Dis* 2012, **12**(4):330-340.

22. Harrington WE, Kakuru A, Jagannathan P: **Malaria in pregnancy shapes the development of foetal and infant immunity**. *Parasite Immunol* 2019, **41**(3):e12573.

23. Kakuru A, Staedke SG, Dorsey G, Rogerson S, Chandramohan D: **Impact of *Plasmodium falciparum* malaria and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants: a systematic review**. *Malar J* 2019, **18**(1):304.

24. Sundell K, Jagannathan P, Huang L, Bigira V, Kapisi J, Kakuru MM, Savic R, Kamya MR, Dorsey G, Aweeka F: **Variable piperaquine exposure significantly impacts protective efficacy of monthly dihydroartemisinin-piperaquine for the prevention of malaria in Ugandan children**. *Malar J* 2015, **14**:368.

25. Klein SL, Flanagan KL: **Sex differences in immune responses**. *Nature Reviews Immunology* 2016, **16**:626.

26. Muenchhoff M, Goulder PJ: **Sex differences in pediatric infectious diseases**. *J Infect Dis* 2014, **209 Suppl 3**:S120-126.

27. Mondal D, Galloway TS, Bailey TC, Mathews F: **Elevated risk of stillbirth in males: systematic review and meta-analysis of more than 30 million births**. *BMC Med* 2014, **12**:220.

28. Goldenberg RL, Andrews WW, Goepfert AR, Faye-Petersen O, Cliver SP, Carlo WA, Hauth JC: **The Alabama Preterm Birth Study: umbilical cord blood *Ureaplasma urealyticum* and *Mycoplasma hominis* cultures in very preterm newborn infants**. *Am J Obstet Gynecol* 2008, **198**(1):43.e41-45.

29. Fish EN: **The X-files in immunity: sex-based differences predispose immune responses**. *Nat Rev Immunol* 2008, **8**(9):737-744.

30. Clifton VL: **Review: Sex and the human placenta: mediating differential strategies of fetal growth and survival**. *Placenta* 2010, **31 Suppl**:S33-39.

31. Prahl M, Jagannathan P, McIntyre TI, Auma A, Wamala S, Nalubega M, Musinguzi K, Naluwu K, Sikyoma E, Budker R *et al*: **Sex disparity in cord blood FoxP3(+) CD4 T regulatory cells in infants exposed to malaria in utero**. *Open Forum Infect Dis* 2017, **4**(1):ofx022.

Figure Legends

Figure 1. Trial profile

SP=sulfadoxine-pyrimethamine, DP=dihydroartemisinin-piperaquine

Figure 2. Time to first episode of malaria stratified by infant sex

Panel A= males, panel B= females

IPTp= Intermittent preventive treatment of malaria in pregnancy, SP=sulfadoxine-pyrimethamine, DP=dihydroartemisinin-piperaquine.

Table 5.1 Characteristics of study participants and their mothers

Characteristic	Maternal IPTp arm		p-value
	Monthly SP (N=339)	Monthly DP (N=339)	
Maternal characteristics at enrolment			
Age in years, mean (SD)	23.9 (5.9)	23.9 (5.7)	0.98
Gravidity, n (%)			
Primigravida	86 (25.4%)	73 (21.5)	
Secundigravida	72 (21.2%)	90 (26.6)	0.21
Multigravida	181 (53.4%)	176 (51.8)	
Gestation age categories, n (%)			
12-16 weeks	195 (57.5%)	204 (60.2%)	0.48
>16-20 weeks	144 (42.5%)	135 (39.8%)	
Parasite prevalence by microscopy, n (%)	170 (50.2%)	183 (54.0%)	0.32
Maternal characteristics during pregnancy			
Parasite prevalence by microscopy[a], n/N (%)	828/2291 (36.1%)	365/2324 (15.7%)	<0.001
Incidence of malaria (episodes/PPY)	0.59	0.09	<0.001
Measures of placental malaria			
Placental blood positive by microscopy[b], n/N (%)	29/326 (8.9%)	1/331 (0.3%)	<0.001
Placental blood positive by LAMP[c], n/N (%)	71/320 (22.2%)	7/329 (2.1%)	<0.001
Positive placental histology[d], n/N (%)	199/327 (60.9%)	93/329 (28.3%)	<0.001
Characteristics of infants at birth			
Preterm birth, n (%)	27 (8.0%)	17 (5.0%)	0.12
Gestation age in weeks, mean (SD)	39.4 (1.9)	39.6 (1.6)	0.08
Low birth weight, n (%)	34 (10.0%)	26 (7.7%)	0.28
Birth weight in grams, mean (SD)	3052 (505)	3023 (408)	0.41
Female sex, n (%)	166 (49.0%)	180 (53.1%)	0.28

Abbreviations: DP dihydroartemisinin-piperaquine, IPTp intermittent preventive treatment of malaria in pregnancy, LAMP loop-mediated isothermal amplification, PPY per person year, SD standard deviation, SP sulfadoxine-pyrimethamine

[a]Defined as number of positive blood smears during routine visits divided by total number of routine blood smears

[b] Defined as detection of parasites in placental blood by microscopy

[c] Defined as detection of parasites in placental blood by LAMP

[d]Defined as detection of parasites or pigment in placental tissue

Table 5.2 Impact of IPTp on the incidence of malaria during infancy stratified by sex, age, and gravidity

Strata		Maternal IPTp arm	Number of infants	Episodes of malaria	Person years of follow-up	Incidence of malaria (PPY)	IRR (95% CI)	p-value
All infants born alive		SP	339	602	304.4	1.98	0.87 (0.73-1.03)	0.11
		DP	339	529	309.2	1.71		
Sex	Female infants	SP	166	283	152.5	1.86	0.99 (0.79-1.24)	0.93
		DP	180	303	164.8	1.84		
	Male infants	SP	173	319	151.8	2.10	0.75 (0.58-0.98)	0.03
		DP	159	226	144.3	1.57		
Female infants stratified by age	0-3 months of age	SP	166	30	40.1	0.75	1.16 (0.72-1.89)	0.54
		DP	180	38	43.6	0.87		
	>3-12 months of age	SP	156	253	112.5	2.25	0.97 (0.77-1.24)	0.83
		DP	168	265	121.3	2.19		
Male infants stratified by age	0-3 months of age	SP	173	22	41.5	0.53	0.89 (0.48-1.66)	0.72
		DP	159	18	38.2	0.47		
	>3-12 months of age	SP	160	297	110.3	2.69	0.73 (0.56-0.96)	0.02
		DP	145	208	106.1	1.96		
Maternal gravidity	1	SP	86	131	69.3	1.89	0.84 (0.58-1.20)	0.33
		DP	73	102	64.6	1.58		
	2	SP	72	97	65.5	1.48	1.04 (0.72-1.49)	0.85
		DP	90	124	81.1	1.53		
	$\geq$ 3	SP	181	374	169.5	2.21	0.84 (0.67-1.07)	0.15
		DP	176	303	163.5	1.85		

Abbreviations: CI confidence interval, DP dihydroartemisinin-piperaquine, IPTp intermittent preventive treatment of malaria in pregnancy, IRR incidence rate ratio, PPY per person year, SP sulfadoxine-pyrimethamine

Table 5.3 Secondary outcomes

Incidence measures	Maternal IPTp arm	Number of cases (incidence PPY)	IRR (95% CI)	p-value
Complicated malaria	SP	44 (0.145)	0.54 (0.32-0.92)	0.02
	DP	24 (0.078)		
All cause hospitalisations	SP	19 (0.062)	0.39 (0.15-1.05)	0.06
	DP	8 (0.026)		
Infant mortality	SP	9 (0.030)	0.45 (0.03-7.88)	0.59
	DP	7 (0.023)		
Non-malarial febrile illnesses	SP	1022 (3.36)	1.01 (0.91-1.12)	0.87
	DP	1047 (3.39)		
Prevalence measures[a]	**Maternal IPTp arm**	**Prevalence (%)**	**PR (95% CI)**	**p-value**
Parasitaemia[b]	SP	344/3879 (8.9%)	1.02 (0.83-1.27)	0.84
	DP	357/3933 (9.1%)		
Anaemia[c]	SP	222/878 (25.3%)	0.96 (0.79-1.17)	0.70
	DP	216/892 (24.2%)		

Abbreviations: CI confidence interval, DP dihydroartemisinin-piperaquine, IPTp intermittent preventive treatment of malaria in pregnancy, IRR incidence rate ratio, PPY per person year, PR prevalence ratio, SP sulfadoxine-pyrimethamine

[a]Prevalence measures are period prevalence

[b]Proportion of blood smears with parasitaemia measured routinely every 4 weeks starting at 4 weeks of age

[c] Defined as proportion with haemoglobin < 10 gm/dL measure routinely at 12, 28, and 52 weeks of age

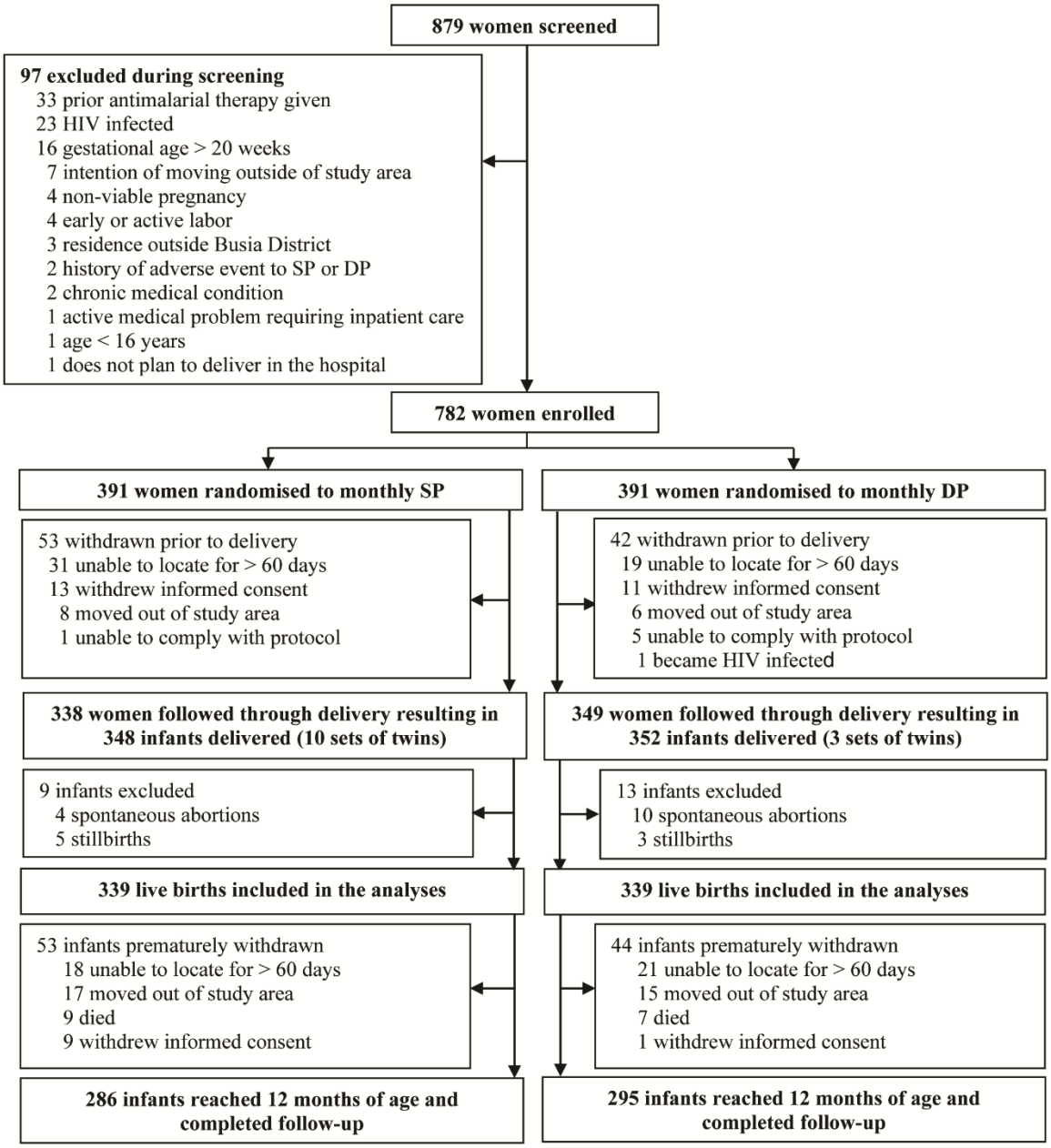

Figure 5.1 Trial profile

SP, sulfadoxine-pyrimethamine; DP, dihydroartemisinin-piperaquine

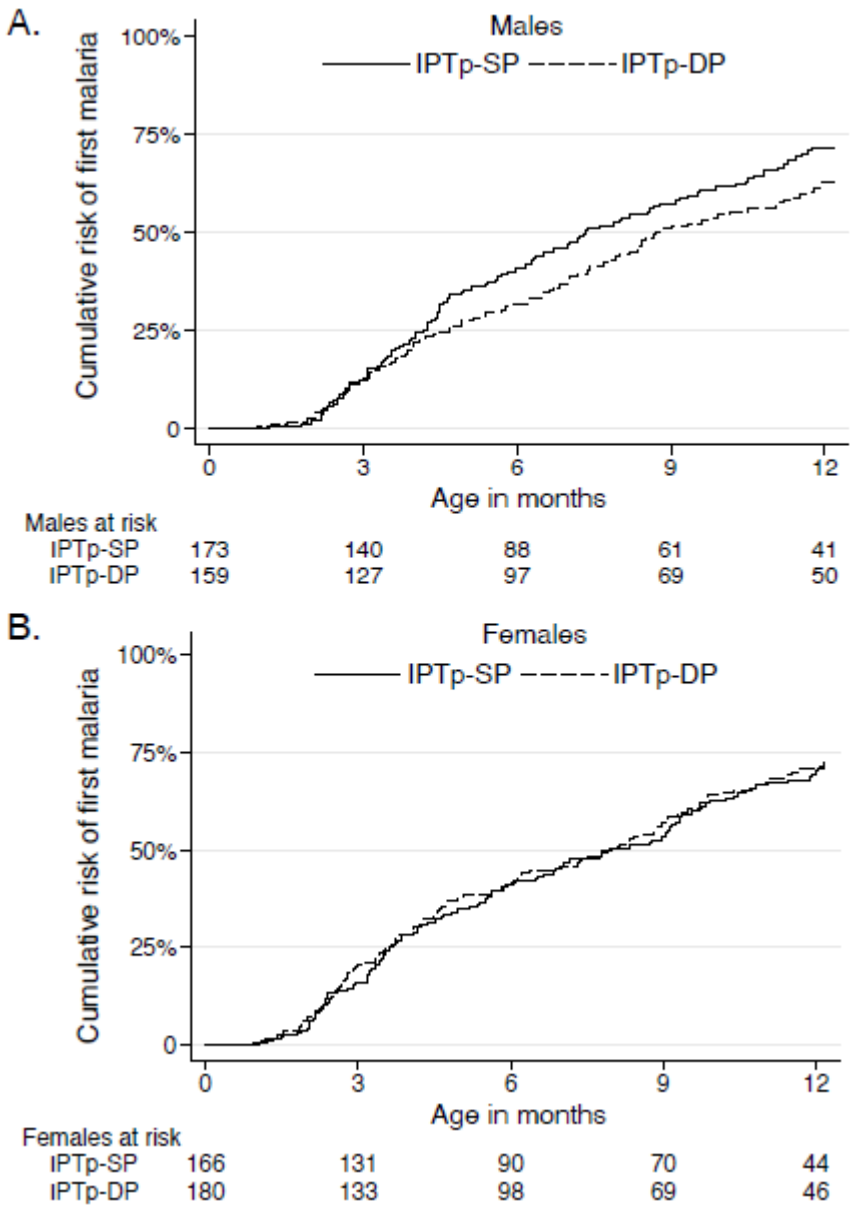

Figure 5.2 Time to first episode of malaria

IPTp, intermittent preventive treatment of malaria in pregnancy; SP, sulfadoxine-pyrimethamine; DP, dihydroartemisinin-piperaquine

CHAPTER 6 PLACENTAL MALARIA AND INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY ON CORD BLOOD *P. FALCIPARUM* IGG ANTIBODY LEVELS

6.1 Chapter introduction

This chapter presents a manuscript which addresses Objective 3, to evaluate the effect of PM and IPTp on cord blood levels of IgG antibodies against *P. falciparum* blood stage antigens. Antibody levels specific to antigens; AMA1, EBA140, EBA175, EBA181, GLURP, MSP1, Rh2, Rh4, and Rh5 levels were measured in maternal and cord blood samples collected from mothers who participated in the main trial, a double-blind, randomised, controlled trial of monthly IPTp with DP versus SP. Maternal and cord blood levels of specific *P. falciparum* IgG antibodies were compared among infants born to mothers with PM and those born to mothers with no PM, and among infants born to mothers who received IPTp-DP and those born to mothers who received IPTp-SP. This manuscript will be submitted to the BMC Immunology journal for consideration for publication.

6.2 Research paper

On the next two pages is the manuscript cover page followed by the manuscript, tables, and figures.

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1602857	**Title**	Dr
First Name(s)	Abel		
Surname/Family Name	Kakuru		
Thesis Title	Impact of malaria in pregnancy and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants		
Primary Supervisor	Sarah G. staedke		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	BMC Immunology
Please list the paper's authors in the intended authorship order:	Abel Kakuru, Nicholas Zehner, Richard Kajubi, Sarah G. Staedke, Daniel Chandramohan, Moses R Kamya, Grant Dorsey, Isaac Ssewanyana, Prasanna Jagannathan

Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I had input in study conception, participated in obtaining study IRB approvals, coordinated and participated in data collection, conducted data analysis, and wrote the first draft of the manuscript

SECTION E

Student Signature	[redacted]
Date	21/09/2020

Supervisor Signature	[redacted]
Date	21 Sept 2020

Association between placental malaria, intermittent preventive treatment of malaria in pregnancy and maternal-foetal transfer of antibodies to *P. falciparum*

Abel Kakuru[1,2], Nicholas Zehner[3], Richard Kajubi[2], Sarah G. Staedke[1], Daniel Chandramohan[1], Moses R Kamya[4], Grant Dorsey[5], Isaac Ssewanyana[2] Prasanna Jagannathan[3]

[1]London School of Hygiene and Tropical Medicine, London, UK

[2]Infectious Diseases Research Collaboration, Kampala, Uganda

[3]Stanford University, Stanford, USA

[4]School of Medicine, Makerere University College of Health Sciences, Kampala, Uganda

[5]Department of Medicine, University of California, San Francisco, USA

Key words: Placental malaria, *Plasmodium falciparum,* maternal-foetal, Immunoglobulin G

Corresponding author: Abel Kakuru, Infectious Diseases Research Collaboration, 2C Nakasero Hill Road, Kampala, Uganda. Email: akakuru@idrc-uganda.org

Abstract

Background

Infants born to mothers with placental malaria (PM) have been reported to have a higher risk of malaria than those born to mothers without PM, which may be due to reduced maternal-foetal transfer of immunity against *Plasmodium falciparum, where* immunoglobulin G (IgG) antibodies play a critical role. To explore this further, *P. falciparum* IgG antibody levels were measured in samples collected at delivery from infants born to HIV-uninfected pregnant mothers who participated in a double-blind, randomised controlled trial of monthly intermittent preventive treatment of malaria in pregnancy (IPTp) with dihydroartemisinin-piperaquine (DP) versus sulfadoxine pyrimethamine (SP).

Methods

Maternal and cord blood IgG antibody levels to *P. falciparum* antigens apical membrane antigen-1 (AMA1), erythrocyte binding antigen-140 (EBA140), EBA175, EBA181, glutamate rich protein (GLURP), merozoite surface protein-1 (MSP1), reticulocyte-binding protein homologue-2 (Rh2), Rh4, and Rh5 were measured in 600 paired samples. Antibody levels and ratios of antibodies in maternal and cord blood samples were compared between mothers who had active PM, mild-moderate PM, and severe past PM and those who had no PM; and between mothers randomised to receive IPTp-DP and those to IPTp-SP. The association between *P. falciparum* IgG antibody levels in cord blood and the incidence of malaria during infancy was assessed using negative binomial regression.

Results

From December 9, 2016 to December 5, 2017, 600 maternal and cord blood samples were collected and analysed. There were no significant differences in cord blood antibody levels to AMA1, EBA140, EBA175, EBA 181, MSP1, Rh2, Rh4 and Rh5 among samples of infants born mothers with active PM, mild-moderate past PM and severe past PM compared to those from infants born to mothers with no PM. Cord to maternal antibody ratios were similar among mothers in different categories of PM. Compared to IPTp-SP, cord blood levels of most measured IgG *P. falciparum* antibodies were similar except for Rh4 which was significantly lower in cord blood of infants born to mothers who received IPTp-DP compared to those who received IPTp-SP. Lower cord blood levels of specific *P. falciparum* IgG antibodies were not associated with a higher incidence of malaria or higher rate of first malaria episode during infancy.

Conclusion

Placental malaria was not associated with lower levels of IgG antibodies to *P. falciparum* antigens in cord blood. Similarly, effective IPTp with DP did not affect levels of most IgG antibodies in cord blood as compared to IPTp-SP. This suggests that PM and IPTp do not affect maternal-foetal transfer of antimalarial antibodies and that other factors might explain the increased risk of malaria in infancy among infants born to mothers with PM.

Background

During pregnancy, maternal immunoglobulin G (IgG) antibodies are actively transferred across the placenta to the foetus [1]. The transferred IgG antibodies are thought to provide the newborn with protection against infectious diseases during early life [2-4]. In sub-Saharan Africa, where most areas are endemic for *Plasmodium falciparum*, most pregnant women are exposed to *P. falciparum* infection [5] which may lead to placental malaria (PM). Placental *P. falciparum* infection is characterised the presence of parasites in intervillous spaces, infiltration of inflammatory cells in intervillous spaces, and thickening of the basement membrane [6, 7]. These pathological changes have been associated with adverse birth outcomes, including preterm births, low birth weight, and stillbirths [8-10]. Placental malaria is also thought to affect the maternal-foetal transfer of IgG antibodies to common infectious disease-causing agents. Several studies have reported an association between PM and reduced maternal-foetal transfer of IgG antibodies to common infectious diseases, including measles [11], tetanus [12], and malaria [13], which may impact on infant immunity to infectious diseases early in life. Indeed, infants born to mothers with PM have been reported to have a higher risk of malaria in the first months of life than infants born to mothers without PM [14, 15], but it is not well known whether this increased risk can be explained by reduced levels of *P. falciparum* IgG antibodies in cord blood.

Intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) remains one of the main interventions recommended by the World Health Organization for preventing PM and improving birth outcomes [16]. However, widespread antifolate resistance of *P. falciparum* in East and Central Africa is threatening the effectiveness of IPTp-SP [17]. Studies evaluating alternative drugs for IPTp have shown dihydroartemisinin-piperaquine (DP) to be a promising alternative. Compared to IPTp-SP, IPTp-DP was associated with reduced risk of clinical malaria during pregnancy, parasitaemia during pregnancy, and PM at delivery [18-20]. We recently reported that infants born to mothers who received IPTp-DP had a reduced risk of malaria during the first year of life compared to infants born to mothers who received IPTp-SP, but this association was only observed in male infants [21]. Whether this reduced risk of malaria in infants born to mothers who received IPTp-DP can be explained by a greater maternal-foetal transfer of antimalarial antibodies is not known.

To evaluate the association between PM, IPTp and *P. falciparum* IgG antibody levels in cord blood we compared cord blood *P. falciparum* antibody levels, and the ratio of cord and maternal blood antimalarial antibodies among infants born to mothers with PM to those born to mothers

without PM, and among infants born to mothers who received IPTp-DP to those born to mothers who received IPTp-SP.

Methods

Study design and collection of samples

This study was part of a double-blind, randomised, controlled trial of monthly IPTp with DP versus SP in HIV-uninfected pregnant women and their infants conducted in Busia District, Uganda, between September 2016- December 2018. Details of the study have been previously described [19, 21, 22]. In brief, HIV-uninfected pregnant women were enrolled at 12-20 weeks of gestation and randomised to receive monthly DP vs SP. Women were reviewed monthly for routine assessment and administration of study drugs. Pregnant women were followed up through delivery. At delivery, maternal blood, cord blood, placental blood, and placental tissue were collected. Maternal and cord blood samples were collected in EDTA tubes, centrifuged, and the plasma was stored at -80°C. Infants born alive were followed-up to 12 months of age in a dedicated study clinic for all their medical and were reviewed monthly for routine assessment. Infants who presented to the study clinic with a history of fever in the past 24 hours or with a documented tympanic temperature of $\geq$ 38.0˚C had an urgent thick blood smear done for detection of malaria parasites. Infants with a fever and a positive thick blood smear were diagnosed with malaria and treated according to the Uganda Ministry of Health guidelines.

Diagnosis of placental malaria

Placental malaria was diagnosed from placental blood by microscopy and loop-mediated isothermal amplification (detection of parasites), and from placental tissue by histology (detection of parasites or malaria pigment) as described previously [23, 24]. Severity of malaria was quantified by the proportion of high-power fields (HPF) with malaria pigment deposition in placental fibrin.

Measurement of IgG antibodies in maternal and cord blood

Levels of antibodies to specific *P. falciparum* antigens were measured on stored maternal and cord plasma samples collected at delivery using a multiplex antibody bead assay. Luminex bead conjugation was performed as previously described [25]. Thawed plasma (50 µl), in duplicate (1/200 dilution), was co-incubated with microsphere mixtures on a 96-well plate for one hour, washed, stained, and incubated with a secondary antibody, then washed and read by the MAGPIX system. Antibody levels were expressed in arbitrary units (AUs), calculated by dividing the median fluorescence intensity (MFI) of the sample by the MFI plus 3 standard deviations

(SD) of samples from Europeans never exposed to malaria. Positive control samples from individuals with known antibodies to these antigens were pooled, and standard curves were generated through serial dilutions of the positive control pool. The antibodies measured included those to specific *P. falciparum* antigens that are expressed at the erythrocytic stage of the parasite life cycle, that have been shown to be protective against malaria or are promising vaccine candidates. These included apical membrane antigen-1 (AMA1) [26], erythrocyte binding antigen-140 (EBA140), EBA175, EBA181 [27], glutamate rich protein (GLURP) [26], merozoite surface protein-1 (MSP1) [28], reticulocyte binding protein homologue-2 (Rh2), Rh4, and Rh5 [29-32].

Statistical analyses

Data were double entered and verified into an Access database and analyses were conducted using Stata version 14.2. PM was categorised into four categories 1) no PM (no evidence of parasites or pigment), 2) active PM (evidence of parasites with or without pigment), 3) mild-moderate past PM (no parasites detected but with <=20% of high-power fields with pigment deposition in fibrin), and 4) severe past PM (>20% of HPF with pigment deposition in fibrin with no parasites detected).

The means of log transformed *P. falciparum* antibody levels in maternal and cord blood were compared among infants born to mothers randomised to receive IPTp-DP versus SP and among infants born to mothers with active PM, mild-moderate past PM, severe past PM versus no PM using linear regression and adjusted for maternal parasitaemia at enrolment, gravidity, maternal IPTp, and household type (modern house built with cement with nets in vents versus traditional house built with mud and wattle with open eaves).

The association between cord blood *P. falciparum* IgG antibody levels and the incidence of malaria during the first year of life was assess using negative binomial regression. First the level of each specific IgG antibody in cord was fitted in the model as a continuous exposure variable to assess for a possible linear relationship. Because there was no linear relationship observed for any specific IgG antibody with the incidence of malaria during infancy, antibody levels were stratified into three subpopulations using percentiles; 1) ≤25th percentile, 2) >25-50th percentile and 3) >50th percentile. To increase statistical power, the >50-75% and the >75% categories were combined because the association between level of antibodies and the incidence of malaria had similar effect sizes comparing the >50-75% category with the ≤25% category, and the >75% category with the ≤25% category. The incidence of malaria during the first year of life among infants with specific *P. falciparum* IgG antibody levels in cord blood >25-50th percentile and >50th percentile, was then compared with incidence of malaria among infants with IgG antibody levels

≤25th percentile adjusting for maternal IPTp arm, maternal parasitaemia at baseline, gravidity, and household type.

To reduce the probability of type I error due to multiple hypothesis tests conducted, the level of significance was reduced by dividing the p-value of 0.05 by the number of hypothesis tests conducted, to p<0.002 (0.05/27) for the associations between IgG levels and PM categories, p<0.006 (0.05/9) for the associations between IgG levels and IPTp, and p <0.003 (0.05/18) for the associations between levels of IgG and the incidence of malaria during the first year of life.

Results

Cohort description

Of the 782 HIV-uninfected pregnant women enrolled, 687 were followed through delivery resulting in 678 live births, 339 born to mothers on monthly IPTp-DP and 339 born to mothers receiving IPTp-DP [21] (Figure 6.1). A total of 600 infants (297 born to mothers receiving monthly IPTp-SP, and 303 born to mothers receiving monthly IPTp-DP) had cord blood collected at delivery of which 584 were followed up to one year. Maternal characteristics at enrolment were similar between the two treatment arms (Table 6.1). At enrolment, 287 (48%) mothers were primigravidae or secundigravidae, 461 (77%) were residing in traditional houses, and 82% of mothers had parasitaemia detected by microscopy or qPCR at enrolment.

Association between placental malaria and *P. falciparum* IgG antibodies in maternal and cord blood at delivery

Overall, 600 cord blood samples were collected and analysed for levels of *P. falciparum* IgG antibodies. *P. falciparum* IgG antibodies were measurable in all cord blood samples. There were strong positive correlations between levels of specific *P. falciparum* IgG antibodies in maternal blood and levels of the same antibodies in cord blood (Table 6.2, Figure 6.2).

In the analysis adjusted for maternal IPTp, gravidity, maternal parasitaemia at enrolment and household type, mothers with active PM (mean log MFI 10.04, SD 0.89, p=0.03), mild-moderate past PM (mean log MFI 10.03, SD 0.08, p=0.02) and severe past PM (mean log MFI 10.19, SD 0.43, p=0.01) had higher levels of AMA1 antibodies compared to mothers with no PM (Table 6.3), but the difference was not statistically significant at the level of p<0.002. Similarly, compared to no PM (mean log MFI 7.98, SD 1.37), active PM (mean log MFI 8.48, SD 1.35, p=0.03) and severe past PM (mean log MFI 8.63, SD 1.35, p=0.02) were associated with higher maternal levels of Rh2 antibodies but the difference was not statically significant. Maternal

levels of GLURP antibodies were lower in mothers with severe past PM (mean log MFI 8.16, SD 1.74) compared to those with no PM (mean log MFI 9.45, SD 1.56, p=0.03). There were no significant differences in maternal IgG antibody blood levels of EBA140, EBA175, EBA181, MSP1, Rh4, and Rh5 antibodies.

Infants born to mothers with severe past PM had lower levels of antibodies to GLURP (mean log MFI 8.58, SD 1.85) than those born to mothers with no PM (mean log MFI 9.52, SD 1.24, p=0.006; Table 6.3). There were no significant differences in cord blood levels of AMA1, EBA140, EBA175, EBA181, MSP1, Rh4, and Rh5 antibodies among infants born to mothers with active PM, mild-moderate past PM, and severe past PM compared to those born to mothers without PM (Table 6.3). Cord to maternal blood ratios of all antibodies tested were similar among mothers with active PM, mild-moderate past PM, and severe past PM compared to mothers with no PM (Table 6.3) and this relationship did not differ between male and female infants.

Association between IPTp and *P. falciparum* IgG levels in maternal and cord blood at delivery

We next considered the impact of different IPTp regimens on maternal IgG antibody levels. Maternal antimalarial antibody levels of Rh2, Rh4, and Rh5 were lower among mothers who received IPTp-DP compared to those who received IPTp-SP, but the difference was not statistically significant (Table 6.4). There were no significant differences in maternal antibody levels to AMA1, EBA140, EBA175, EBA181, GLURP, and MSP1 among mothers receiving monthly IPTp-DP compared to those receiving monthly IPTp-SP (Table 6.4, Figure 6.3), although the maternal IgG antibody levels for most antimalarial antibodies were slightly lower for mothers who received IPTp-DP compared to those who received IPTp-SP.

There were no significant differences in cord blood levels of AMA1, EBA140, EBA175, EBA181, GLURP, MSP1, Rh2, and Rh5 among infants born to mothers who received IPTp-DP compared to those born to mothers who received IPTp-SP (Table 6.4). Cord blood levels of Rh4 antibodies were significantly lower in infants born to mothers who received IPTp-DP (mean log MFI 6.43, SD 1.49; Table 6.4) compared to mothers who received IPTp-SP (mean log MFI 6.81, SD 1.53, p=0.002, Figure 6.4). The cord-maternal ratios of all the measured *P. falciparum* IgG antibodies were similar among mothers who received IPTp-DP compared to those who received IPTp-SP. There was no effect modification by infant sex, observed in the association between IPTp and cord blood antimalarial IgG antibody levels.

Association between cord blood antibody levels and malaria risk during the first year of life.

We next assessed for the association between specific *P. falciparum* IgG antibody levels in cord blood and the incidence of malaria during the first 12 months of life. There was no significant

association between IgG antibody levels and the incidence of malaria during the first 12 months of life at the level of p<0.003. Compared to IgG antibody levels within the 25th percentile, levels of antibodies above the 50th percentile to AMA1, EBA175, and Rh5 were associated with a higher incidence of malaria during infancy, after adjustment for maternal parasitaemia at enrolment, maternal gravidity, IPTp arm, and household type (AMA1 (Incidence rate ratio [IRR] 1.25, 95% confidence interval [CI] 1.03-1.59, p=0.02; Table 6.5), EBA175 (IRR 1.27, 95% CI 1.03-1.57, p=0.03), and Rh5 (IRR 1.30, 95% CI 1.06-1.60, p=0.01)) but the difference was not statistically significant (significance level p<0.003). No effect modification by infant sex was observed in the association between antimalarial antibody levels in cord blood and the incidence of malaria in infants. There were no significant differences in the rates of first malaria episode among infants with cord blood antimalarial antibody levels >25th -50th percentile or >50th percentile and infants with cord blood antimalarial antibody levels ≤ 25th percentile (Table 6.5)

Discussion

Maternally transferred antimalarial IgG antibodies may be important in providing protection against malaria in the infant during early life [33]. We evaluated for associations between PM, IPTp and levels of *P. falciparum* IgG antibodies in maternal and cord blood by comparing levels of *P. falciparum* IgG antibodies and foetal to maternal cord blood ratios among infants born to mothers with active PM, mild-moderate past PM, and severe past PM compared to those born to mothers with no PM; and among infants born to mothers who received monthly IPTp-DP vs those born to mothers who received IPTp-SP. Overall, there was no evidence of a difference in cord blood levels of most measured *P. falciparum* IgG antibodies in infants born to mothers with active PM, past PM (mild-moderate past PM and severe past PM) compared to infants born to mothers with no PM. Cord to maternal antibody ratios were similar among mothers with active PM, mild-moderate past PM, and severe past PM compared to those with no PM. There was no evidence of a difference in cord blood *P. falciparum* antibody levels (AMA1, EBA140, EBA175, EBA181, GLURP, MSP1, Rh2, Rh4, and Rh5) between infants born to mothers who received IPTp-DP compared to those born to mothers who received IPTp-SP. These data suggest that other factors might explain the increased risk of malaria in infancy among infants born to mothers with placental malaria.

In this study, there were no differences in cord blood levels of *P. falciparum* IgG antibodies measured and cord to maternal blood antibody ratios were similar, between infants born to mothers with active PM, mild-moderate PM, or severe past PM and those born to mothers without PM. This suggests that PM may not affect transfer maternal-foetal transfer of

antimalarial antibodies. Contrary to our study findings, some studies have previously demonstrated that PM was associated with a reduced maternal-foetal transfer of *P. falciparum* IgG antibodies [13, 34]. In a study conducted in Benin, PM was associated with reduced maternal to foetal transfer of IgG antimalarial antibodies to AMA1, MSP, and GLURP antigens [13]. However, similar to our study, the cord blood antimalarial antibody levels were not significantly different between infants born to mothers with PM and those born to mothers with no PM. In Mozambique, maternal parasitaemia (parasites detected in maternal peripheral blood or by PCR or microscopy, or parasites detected in placental tissue by histology or PCR) was associated with a reduced maternal-foetal transfer of IgG1 antimalarial antibodies to AMA1 and parasite lysate but not total IgG to these specific antigens [34] suggesting that maternal malaria may affect the transfer of some specific antimalarial IgG antibody subtypes and not others.

Transfer of maternal IgG antibodies to the foetus is an active process which is mediated through foetal Fc receptors located in the syncytiotrophoblast [1] and PM is thought to reduce this transfer by altering placental integrity [35]. We had hypothesised that highly effective IPTp with DP, which has been shown to substantially reduce the risk of PM [18-20], would be associated with higher levels of antimalarial IgG antibodies in cord blood compared to IPTp-SP, by improving maternal-foetal transfer of antibodies. However, contrary to this hypothesis, in this study, IPTp-DP was not associated with higher antimalarial antibody levels in cord blood compared to IPTp-SP, similar to what was reported previously in a study conducted in Uganda [36]. Furthermore, there were no differences in cord to maternal blood antibody ratios among infants born to mothers who received IPTp-DP compared to those born to mothers who received IPTp-SP, indicating that IPTp-DP did not improve maternal-foetal transfer of antibodies. Our results suggest that in an area of high malaria transmission intensity, effective IPTp may not alter maternal-foetal transfer of antimalarial antibodies.

In Ghana, IPTp was found to be associated with reduced maternal IgG antibodies to *P. falciparum* antigens, specifically GLURP [37], suggesting that IPTp with highly effective drugs may reduce maternal IgG antibody levels to *P. falciparum* antigens. However, in our study, despite IPTp-DP being associated with significant reduction in the risk of clinical malaria and asymptomatic parasitaemia during pregnancy compared to IPTp-SP [19], levels of most of the measured *P. falciparum* specific antibodies at delivery, including GLURP, were similar among mothers who received IPTp-DP compared to those who received IPTp-SP. Only maternal IgG antibody levels to Rh2, Rh4, and Rh5 tended to be lower in mothers who received IPTp-DP possibly indicating reduced exposure to merozoites due to the prophylactic effect of the DP. The difference in the findings of our study and the Ghanaian study could be related to differences in the level of malaria transmission intensity in the study site. Our study was conducted in a very high malaria

transmission setting, supported by a very high baseline malaria parasitaemia [19], while the study in Ghana was conducted in a moderate malaria transmission setting [38]. Our findings suggest that in a setting of high malaria transmission intensity, highly effective IPTp with DP may not reduce maternal IgG antimalarial antibodies compared to IPTp-SP.

Maternal IgG antibodies to *P. falciparum* transferred to the feotus are thought to provide protection against malaria to the infant during the first few months of life [4, 33, 39]. However, in this study, there was no evidence of an association between levels of *P. falciparum* specific IgG antibodies and the incidence of malaria in infants during the first three months of life or during the first 12 months of life similar to what was previously reported in Ghana [40]. In contrast, we observed that cord blood levels of antimalarial antibodies to AMA1, EBA175, and Rh5 greater than the 50th percentile were associated with a trend towards a higher incidence of malaria during the first year of life as compared to cord blood antibody levels within the 25th percentile, suggesting that levels of antimalarial IgG antibodies transferred may instead reflect the level of malaria transmission intensity [2].

The lack of an association between PM, IPTp and maternal-foetal transfer of antimalarial antibodies suggests that altered maternal-foetal transfer of IgG antimalarial antibodies may not explain the observed association between PM, IPTp and the incidence of malaria in infant observed in chapter 4 and chapter 5 [21]. Other factors such as the influence of PM on cell-mediated immunity [41, 42] and acquisition of antimalarial antibodies in the infant [43] following delivery may explain our observed results.

This study had some limitations. First, a limited number of antimalarial IgG antibodies were measured in this study. Although our analysis may not reflect the full spectrum of malaria antigens, the panel we examined much broader than what was evaluated in prior studies [13, 34]. Second, we did not differentiate between IgG subclasses. Although we found no differences in maternal-foetal transfer of antibodies among mothers with active or past PM compared to no PM, it is possible there could be differences in maternal-foetal transfer of different IgG sub-classes. Indeed, PM has been previously reported to be associated with reduced maternal-foetal transfer of IgG1, IgG2, and IgG4 but not IgG3 antibodies to herpes simplex type 1 virus, respiratory syncytial virus, and varicella-zoster virus [44]. Third, in 12% of the infants, cord blood was not collected. This could have introduced bias if the infants who did not have cord blood samples collected were systematically different from the ones whose samples were collected. However, in this study, there were no differences in infants that had cord blood collected and those whose cord blood was not collected suggesting that bias due to missing data is unlikely. Fifth, multiple statistical tests were carried out in this study. It is possible that some of the significant results could be due to chance alone and therefore should be interpreted with

caution. Finally, we did not test the functionality of the antibodies transferred from mother to the newborn. It is possible that other functional features of malaria-specific IgG were impacted by PM and/or IPTp and could be associated with protection from malaria in infancy.

In conclusion, in this study conducted in an area of very high malaria transmission intensity, there was no evidence of an association between PM, IPTp and maternal-foetal transfer of antibodies to specific *P. falciparum* antigens. Furthermore, we did not find any evidence to suggest that lower levels of IgG antibodies to specific *P. falciparum* antigens are associated with a higher risk of malaria during infancy. Future studies should evaluate the impact of PM and IPTp on foetal antimalarial immunity, including cell-mediated immunity and acquisition of antibodies to *P. falciparum* antibodies and whether this modifies the risk of malaria during infancy in areas of different malaria transmission intensities.

References

1. Vidarsson G, Dekkers G, Rispens T: **IgG subclasses and allotypes: from structure to effector functions.** *Front Immunol* 2014, **5:**520.

2. Dobbs KR, Dent AE: ***Plasmodium*** **malaria and antimalarial antibodies in the first year of life.** *Parasitology* 2016, **143:**129-138.

3. Ciobanu AM, Dumitru AE, Gica N, Botezatu R, Peltecu G, Panaitescu AM: **Benefits and Risks of IgG Transplacental Transfer.** *Diagnostics (Basel)* 2020, **10**.

4. Wilson PT, Malhotra I, Mungai P, King CL, Dent AE: **Transplacentally transferred functional antibodies against *Plasmodium falciparum* decrease with age.** *Acta Trop* 2013, **128:**149-153.

5. World Health Organization: **World malaria report 2019** [https://www.who.int/publications-detail/world-malaria-report-2019]. Accessed 21 Mar 2020.

6. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, Hirt R, Cardesa A, Alonso PL: **Placental pathology in malaria: a histological, immunohistochemical, and quantitative study.** *Hum Pathol* 2000, **31:**85-93.

7. Bulmer JN, Rasheed FN, Morrison L, Francis N, Greenwood BM: **Placental malaria. II. A semi-quantitative investigation of the pathological features.** *Histopathology* 1993, **22:**219-225.

8. Walker PG, ter Kuile FO, Garske T, Menendez C, Ghani AC: **Estimated risk of placental infection and low birthweight attributable to *Plasmodium falciparum* malaria in Africa in 2010: a modelling study.** *Lancet Glob Health* 2014, **2:**e460-467.

9. Moore KA, Simpson JA, Scoullar MJL, McGready R, Fowkes FJI: **Quantification of the association between malaria in pregnancy and stillbirth: a systematic review and meta-analysis.** *Lancet Glob Health* 2017, **5:**e1101-e1112.

10. Thompson JM, Eick SM, Dailey C, Dale AP, Mehta M, Nair A, Cordero JF, Welton M: **Relationship between pregnancy-associated malaria and adverse pregnancy outcomes: a systematic review and meta-analysis.** *J Trop Pediatr* 2020, **66:**327-338.

11. Okoko BJ, Wesuperuma LH, Ota MO, Banya WA, Pinder M, Gomez FS, Osinusi K, Hart AC: **Influence of placental malaria infection and maternal hypergammaglobulinaemia on**

materno-foetal transfer of measles and tetanus antibodies in a rural west African population. *J Health Popul Nutr* 2001, **19:**59-65.

12. Cumberland P, Shulman CE, Maple PA, Bulmer JN, Dorman EK, Kawuondo K, Marsh K, Cutts FT: **Maternal HIV infection and placental malaria reduce transplacental antibody transfer and tetanus antibody levels in newborns in Kenya.** *J Infect Dis* 2007, **196:**550-557.

13. Dechavanne C, Cottrell G, Garcia A, Migot-Nabias F: **Placental malaria: decreased transfer of maternal antibodies directed to *Plasmodium falciparum* and impact on the incidence of febrile infections in infants.** *PLoS ONE [Electronic Resource]* 2015, **10:**e0145464.

14. Bardaji A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, Mabunda S, Alonso PL, Menendez C: **Impact of malaria at the end of pregnancy on infant mortality and morbidity.** *J Infect Dis* 2011, **203:**691-699.

15. Le Port A, Watier L, Cottrell G, Ouedraogo S, Dechavanne C, Pierrat C, Rachas A, Bouscaillou J, Bouraima A, Massougbodji A, et al: **Infections in infants during the first 12 months of life: role of placental malaria and environmental factors.** *PLoS ONE [Electronic Resource]* 2011, **6:**e27516.

16. World Health Organization: **WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP).** [http://www.who.int/malaria/publications/atoz/iptp-sp-updated-policy-brief-24jan2014.pdf]

17. Okell LC, Griffin JT, Roper C: **Mapping sulphadoxine-pyrimethamine-resistant *Plasmodium falciparum* malaria in infected humans and in parasite populations in Africa.** *Sci Rep* 2017, **7**.

18. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, Ouma P, Were V, Laserson K, Katana A, et al: **Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial.** *Lancet* 2015, **386:**2507-2519.

19. Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, Ruel T, Opira B, Ochokoru H, Ategeka J, Nayebare P, et al: **Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in**

pregnancy: a double-blind, randomised, controlled, superiority trial. *Lancet* 2019, **393:**1428-1439.

20. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, Opira B, Olwoch P, Ategeka J, Nayebare P, et al: **Dihydroartemisinin-piperaquine for the prevention of malaria in pregnancy.** *N Engl J Med* 2016, **374:**928-939.

21. Kakuru A, Jagannathan P, Kajubi R, Ochieng T, Ochokoru H, Nakalembe M, Clark TD, Ruel T, Staedke SG, Chandramohan D, et al: **Impact of intermittent preventive treatment of malaria in pregnancy with dihydroartemisinin-piperaquine versus sulfadoxine-pyrimethamine on the incidence of malaria in infancy: a randomized controlled trial.** *BMC Med* 2020, **18:**207.

22. Okiring J, Olwoch P, Kakuru A, Okou J, Ochokoru H, Ochieng TA, Kajubi R, Kamya MR, Dorsey G, Tusting LS: **Household and maternal risk factors for malaria in pregnancy in a highly endemic area of Uganda: a prospective cohort study.** *Malar J* 2019, **18:**144.

23. Hopkins H, Gonzalez IJ, Polley SD, Angutoko P, Ategeka J, Asiimwe C, Agaba B, Kyabayinze DJ, Sutherland CJ, Perkins MD, Bell D: **Highly sensitive detection of malaria parasitemia in a malaria-endemic setting: performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda.** *J Infect Dis* 2013, **208:**645-652.

24. Natureeba P, Ades V, Luwedde F, Mwesigwa J, Plenty A, Okong P, Charlebois ED, Clark TD, Nzarubara B, Havlir DV, et al: **Lopinavir/ritonavir-based antiretroviral treatment (ART) versus efavirenz-based ART for the prevention of malaria among HIV-infected pregnant women.** *J Infect Dis* 2014, **210:**1938-1945.

25. Ambrosino E, Dumoulin C, Orlandi-Pradines E, Remoue F, Toure-Balde A, Tall A, Sarr JB, Poinsignon A, Sokhna C, Puget K, et al: **A multiplex assay for the simultaneous detection of antibodies against 15 *Plasmodium falciparum* and *Anopheles gambiae* saliva antigens.** *Malar J* 2010, **9:**317.

26. Fowkes FJ, Richards JS, Simpson JA, Beeson JG: **The relationship between anti-merozoite antibodies and incidence of *Plasmodium falciparum* malaria: a systematic review and meta-analysis.** *PLoS Med* 2010, **7:**e1000218.

27. Richards JS, Stanisic DI, Fowkes FJ, Tavul L, Dabod E, Thompson JK, Kumar S, Chitnis CE, Narum DL, Michon P, et al: **Association between naturally acquired antibodies to**

erythrocyte-binding antigens of *Plasmodium falciparum* and protection from malaria and high-density parasitemia. *Clin Infect Dis* 2010, **51:**e50-60.

28. Jäschke A, Coulibaly B, Remarque EJ, Bujard H, Epp C: **Merozoite surface protein 1 from *Plasmodium falciparum* Is a major target of opsonizing antibodies in individuals with acquired immunity against malaria.** *Clin Vaccine Immunol* 2017, **24**.

29. World Health Organization: **WHO recommendation on intermittent preventive treatment of malaria in pregnancy | RHL** [https://extranet.who.int/rhl/topics/preconception-pregnancy-childbirth-and-postpartum-care/antenatal-care/who-recommendation-intermittent-preventive-treatment-malaria-pregnancy]

30. Healer J, Wong W, Thompson JK, He W, Birkinshaw RW, Miura K, Long CA, Soroka V, Søgaard TMM, Jørgensen T, et al: **Neutralising antibodies block the function of Rh5/Ripr/CyRPA complex during invasion of *Plasmodium falciparum* into human erythrocytes.** *Cell Microbiol* 2019, **21:**e13030.

31. Reiling L, Richards JS, Fowkes FJ, Barry AE, Triglia T, Chokejindachai W, Michon P, Tavul L, Siba PM, Cowman AF, et al: **Evidence that the erythrocyte invasion ligand PfRh2 is a target of protective immunity against *Plasmodium falciparum* malaria.** *J Immunol* 2010, **185:**6157-6167.

32. Reiling L, Richards JS, Fowkes FJ, Wilson DW, Chokejindachai W, Barry AE, Tham WH, Stubbs J, Langer C, Donelson J, et al: **The *Plasmodium falciparum* erythrocyte invasion ligand Pfrh4 as a target of functional and protective human antibodies against malaria.** *PLoS One* 2012, **7:**e45253.

33. Akum AE, Minang JT, Kuoh AJ, Ahmadou MJ, Troye-Blomberg M: ***Plasmodium falciparum* inhibitory capacities of paired maternal-cord sera from south-west province, Cameroon.** *J Trop Pediatr* 2005, **51:**182-190.

34. Moro L, Bardaji A, Nhampossa T, Mandomando I, Serra-Casas E, Sigauque B, Cistero P, Chauhan VS, Chitnis CE, Ordi J, et al: **Malaria and HIV infection in Mozambican pregnant women are associated with reduced transfer of antimalarial antibodies to their newborns.** *J Infect Dis* 2015, **211:**1004-1014.

35. Brabin BJ, Romagosa C, Abdelgalil S, Menendez C, Verhoeff FH, McGready R, Fletcher KA, Owens S, D'Alessandro U, Nosten F, et al: **The sick placenta-the role of malaria.** *Placenta* 2004, **25:**359-378.

36. Jagannathan P, Kakuru A, Okiring J, Muhindo MK, Natureeba P, Nakalembe M, Opira B, Olwoch P, Nankya F, Ssewanyana I, et al: **Dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria during pregnancy and risk of malaria in early childhood: a randomized controlled trial.** *PLoS Med* 2018, **15:**e1002606.

37. Stephens JK, Kyei-Baafour E, Dickson EK, Ofori JK, Ofori MF, Wilson ML, Quakyi IA, Akanmori BD: **Effect of IPTp on *Plasmodium falciparum* antibody levels among pregnant women and their babies in a sub-urban coastal area in Ghana.** *Malar J* 2017, **16:**224.

38. Stephens JK, Ofori MF, Quakyi IA, Wilson ML, Akanmori BD: **Prevalence of peripheral blood parasitaemia, anaemia and low birthweight among pregnant women in a suburban area in coastal Ghana.** *Pan Afr Med J* 2014, **17 Suppl 1:**3.

39. Hogh B, Marbiah NT, Burghaus PA, Andersen PK: **Relationship between maternally derived anti-*Plasmodium falciparum* antibodies and risk of infection and disease in infants living in an area of Liberia, west Africa, in which malaria is highly endemic.** *Infect Immun* 1995, **63:**4034-4038.

40. Riley EM, Wagner GE, Ofori MF, Wheeler JG, Akanmori BD, Tetteh K, McGuinness D, Bennett S, Nkrumah FK, Anders RF, Koram KA: **Lack of association between maternal antibody and protection of African infants from malaria infection.** *Infect Immun* 2000, **68:**5856-5863.

41. Bisseye C, van der Sande M, Morgan WD, Holder AA, Pinder M, Ismaili J: ***Plasmodium falciparum* infection of the placenta impacts on the T helper type 1 (Th1)/Th2 balance of neonatal T cells through CD4(+)CD25(+) forkhead box P3(+) regulatory T cells and interleukin-10.** *Clin Exp Immunol* 2009, **158:**287-293.

42. Harrington WE, Kakuru A, Jagannathan P: **Malaria in pregnancy shapes the development of foetal and infant immunity.** *Parasite Immunol* 2019, **41:**e12573.

43. Bonner PC, Zhou Z, Mirel LB, Ayisi JG, Shi YP, van Eijk AM, Otieno JA, Nahlen BL, Steketee RW, Udhayakumar V: **Placental malaria diminishes development of antibody responses to *Plasmodium falciparum* epitopes in infants residing in an area of western Kenya where *P. falciparum* is endemic.** *Clin Diagn Lab Immunol* 2005, **12:**375-379.

44. Okoko BJ, Wesumperuma LH, Ota MO, Pinder M, Banya W, Gomez SF, McAdam KP, Hart AC: **The influence of placental malaria infection and maternal hypergammaglobulinemia on transplacental transfer of antibodies and IgG subclasses in a rural West African population.** *J Infect Dis* 2001, **184:**627-632.

Figure Legends

Figure 6.1 Study profile

SP=sulfadoxine-pyrimethamine, DP=dihydroartemisinin-piperaquine

Figure 6. 2 Correlations between maternal and cord blood IgG antibody levels for selected *P. falciparum antibodies*

AMA1= Apical membrane antigen-1, EBA175=Erythrocyte-binding antigen, MSP1= Merozoite surface protein-1, Rh5= reticulocyte-binding protein homologue-5

Figure 6.3 Maternal *P. falciparum* IgG antibody levels measured at delivery stratified IPTp arm

AMA1= Apical membrane antigen-1, AU=Arbitrary units, DP= dihydroartemisinin-piperaquine, EBA=Erythrocyte-binding antigen, GLURP=Glutamate-rich protein, IPTp= intermittent preventive treatment of malaria in pregnancy, MSP1= Merozoite surface protein-1, Rh= reticulocyte-binding protein homologue, SP= sulfadoxine-pyrimethamine

**P<0.05

Figure 6.4 Cord *P. falciparum* IgG antibody levels measured at delivery stratified IPTp arm

AMA1= Apical membrane antigen-1, AU=Arbitrary units, DP= dihydroartemisinin-piperaquine, EBA=Erythrocyte-binding antigen, GLURP=Glutamate-rich protein, IPTp= intermittent preventive treatment of malaria in pregnancy, MSP1= Merozoite surface protein-1, Rh= reticulocyte-binding protein homologue, SP= sulfadoxine-pyrimethamine

Table 6.1 Characteristics of study participants

Characteristic	Maternal IPTp arm	
	Monthly SP (N=297)	Monthly DP (N=303)
Maternal characteristics at enrolment		
Age in years, mean (SD)	23.9 (6.0)	23.9 (5.7)
Gravidity, n (%)		
Primigravida/secundigravida	139 (46.8%)	148 (48.8%)
Multigravida	158 (53·2%)	155 (51·2%)
House-hold type, n (%)		
Modern House	70 (23.6%)	69 (22.8%)
Traditional House	227 (76.4%)	234 (77.2%)
Parasite prevalence by microscopy or qPCR, n (%)		
No parasites	49 (16.5%)	58 (19.1%)
Sub-microscopic parasitemia	97 (32.7%)	81 (26.7%)
Microscopic parasitemia	151 (50.8%)	164 (54.1%)
Maternal characteristics during pregnancy		
Parasite prevalence by microscopy, n/N (%)[a]	723/2019 (35.8%)	326/2078 (15.7%)
Incidence of malaria (episodes per person-year)	0.61	0.09
Placental malaria status		
Placental malaria status, n (%)		
No PM	111 (37.6%)	213 (70.8%)
Active PM	62 (21.0%)	5 (1.7%)
Past PM (Mild-moderate pigment)	87 (29.5%)	77 (25.6%)
Past PM (Severe pigment)	35 (11.9%)	6 (2.0%)
Characteristics of infants at birth		
Preterm birth, n (%)	17 (5.7%)	14 (4.6%)
Low birth weight, n (%)	25 (8.4%)	23 (7.6%)
Female sex, n (%)	143 (48.2%)	163 (53.8%)

Abbreviations: DP, dihydroartemisinin-piperaquine; PM, placental malaria; qPCR, quantitative polymerase chain reaction; SD, standard deviation; SP, sulfadoxine-pyrimethamine

[a]Defined as number of routine positive blood smears divided by total number of routine blood smears

No PM=no parasites or pigment detected; active PM=parasites detected with or without pigment

Past PM (Mild-moderate) = >0-20% of high-power fields with pigment but no parasites; Past PM (severe)= >20%-60% of high-power fields with pigment but no parasites

Table 6.2 Correlation between maternal and cord blood IgG antibody levels

Antibody	Mean IgG antibody levels (SD)		Correlation coefficient
	Maternal blood	Cord blood	
AMA1	9.92 (0.77)	10.13 (0.73)	0.62
EBA140	7.25 (1.72)	7.33 (1.78)	0.90
EBA175	8.44 (1.36)	8.54 (1.40)	0.83
EBA181	7.29 (1.28)	7.38 (1.44)	0.80
GLURP	9.30 (1.30)	9.35 (1.37)	0.85
MSP1	8.92 (1.24)	9.17 (1.32)	0.73
Rh2	8.16 (1.38)	8.65 (1.31)	0.83
Rh4	6.58 (1.32)	6.62 (1.52)	0.81
Rh5	6.35 (1.02)	6.43 (1.22)	0.66

AMA1= Apical membrane antigen-1, EBA=Erythrocyte-binding antigen, GLURP=Glutamate rich protein, MSP1= Merozoite surface protein, Rh= reticulocyte-binding protein homologue

Table 6.3 Maternal, cord blood IgG antibody levels at delivery stratified by PM status

P. falciparum IgG antibodies	Specimen type	Placental malaria status						
		No PM[a] Mean (SD), N=323	Active PM Mean (SD), N=67	p-value	Mild-mod past PM Mean (SD), N=164	p-value	Severe past PM Mean (SD), N=41	p-value
AMA1	Maternal	9.79 (0.80)	10.04 (0.89)	0.03	10.03 (0.08)	0.02	10.19 (0.43)	0.01
	Cord	10.02 (0.83)	10.20 (0.86)	0.15	10.26 (0.43)	0.07	10.32 (0.31)	0.12
	Cord/Maternal	1.03 (0.08)	1.03 (1.00)	0.80	1.03 (0.09)	0.19	1.01 (0.02)	0.42
EBA140	Maternal	7.26 (1.74)	7.47 (1.70)	0.35	7.17 (1.71)	0.99	7.14 (1.69)	0.99
	Cord	7.35 (1.84)	7.45 (1.81)	0.78	7.25 (1.70)	0.75	7.22 (1.64)	0.68
	Cord/Maternal	1.02 (0.11)	1.01 (0.22)	0.57	1.02 (0.14)	0.58	1.02 (0.11)	0.56
EBA175	Maternal	8.41 (1.39)	8.50 (1.49)	0.29	8.50 (1.24)	0.15	8.25 (1.33)	0.79
	Cord	8.51 (1.50)	8.48 (1.51)	0.78	8.67 (1.17)	0.17	8.30 (1.34)	0.77
	Cord/Maternal	1.02 (0.12)	1.02 (0.24)	0.76	1.03 (0.12)	0.87	1.01 (0.08)	0.34
EBA181	Maternal	7.36 (1.33)	7.36 (1.14)	0.74	7.15 (1.24)	0.21	7.18 (1.31)	0.49
	Cord	7.46 (1.50)	7.33 (1.36)	0.35	7.30 (1.40)	0.29	7.23 (1.30)	0.37
	Cord/Maternal	1.02 (0.13)	1.01 (0.23)	0.70	1.02 (0.12)	0.93	1.02 (0.13)	0.82
GLURP	Maternal	9.45 (1.56)	9.14 (1.38)	0.37	9.21 (1.32)	0.88	8.61 (1.74)	0.03
	Cord	9.52 (1.24)	9.11 (1.51)	0.13	9.27 (1.37)	0.49	8.58 (1.85)	0.006
	Cord/Maternal	1.01 (0.08)	1.01 (0.21)	0.61	1.01 (0.15)	0.41	1.00 (0.10)	0.21
MSP1	Maternal	8.97 (1.15)	8.82 (1.46)	0.60	8.94 (1.14)	0.34	8.53 (1.79)	0.29
	Cord	9.20 (1.32)	9.16 (1.41)	0.78	9.21 (1.17)	0.46	8.77 (1.72)	0.17
	Cord/Maternal	1.03 (0.12)	1.07 (0.33)	0.15	1.04 (0.10)	0.79	1.04 (0.12)	0.92
Rh2	Maternal	7.98 (1.37)	8.48 (1.35)	0.03	8.28 (1.37)	0.12	8.63 (1.35)	0.02
	Cord	8.48 (1.36)	8.91 (1.21)	0.08	8.74 (1.27)	0.43	9.06 (1.18)	0.09
	Cord/Maternal	1.08 (0.16)	1.07 (0.19)	0.41	1.06 (0.11)	0.09	1.06 (0.12)	0.19
Rh4	Maternal	6.64 (1.38)	6.60 (1.27)	0.61	6.56 (1.26)	0.98	6.30 (1.32)	0.25
	Cord	6.64 (1.60)	6.60 (1.34)	0.47	6.69 (1.47)	0.52	6.23 (1.36)	0.15
	Cord/Maternal	1.00 (0.15)	1.02 (0.23)	0.90	1.02 (0.14)	0.59	1.00 (0.14)	0.56
Rh5	Maternal	6.33 (1.04)	6.48 (0.99)	0.72	6.36 (0.95)	0.73	6.18 (1.13)	0.31
	Cord	6.36 (1.24)	6.55 (1.22)	0.55	6.53 (1.16)	0.35	6.32 (1.32)	0.65
	Cord/Maternal	1.01 (0.16)	1.02 (0.16)	0.78	1.03 (0.14)	0.61	1.03 (0.17)	0.64

[a]The No PM group is the reference group for all p-values calculated. All p-values adjusted for maternal IPTp arm, gravidity, maternal parasite status at enrolment, and house type.

AMA1= Apical membrane antigen-1, EBA=Erythrocyte-binding antigen, GLURP=Glutamate rich protein, MSP1= Merozoite surface protein, Rh= reticulocyte-binding protein homologue

Table 6.4 Comparison of cord blood IgG antibody levels in infants born to mothers on different maternal IPTp arm

IgG antibody	IgG antibody levels			
	Blood sample type	IPTp-SP[a] Mean (SD), N=297	IPTp-DP Mean (SD), N=302	p-value
AMA1	Maternal	9.94 (0.79)	9.89 (0.76)	0.41
	Cord	10.12 (0.83)	10.13 (0.61)	0.85
	Cord/maternal	1.02 (0.13)	1.02 (0.07)	0.67
EBA140	Maternal	7.34 (1.77)	7.16 (1.66)	0.21
	Cord	7.43 (1.85)	7.23 (1.70)	0.16
	Cord/maternal	1.02 (0.15)	1.01 (0.12)	0.54
EBA175	Maternal	8.48 (1.36)	8.39 (1.35)	0.41
	Cord	8.57 (1.45)	8.52 (1.35)	0.66
	Cord/maternal	1.02 (0.16)	1.02 (0.11)	0.87
EBA181	Maternal	7.38 (1.27)	7.20 (1.28)	0.08
	Cord	7.45 (1.46)	7.32 (1.42)	0.26
	Cord/Maternal	1.02 (0.16)	1.02 (0.12)	0.71
GLURP	Maternal	9.31 (1.33)	9.28 (1.25)	0.71
	Cord	9.34 (1.46)	9.35 (1.28)	0.98
	Cord/Maternal	1.01 (0.16)	1.01 (0.07)	0.95
MSP1	Maternal	8.96 (1.29)	8.88 (1.19)	0.43
	Cord	9.25 (1.39)	9.09 (1.25)	0.16
	Cord/Maternal	1.04 (0.20)	1.03 (0.10)	0.26
Rh2	Maternal	8.29 (1.37)	8.03 (1.38)	0.02
	Cord	8.75 (1.33)	8.54 (1.29)	0.06
	Cord/maternal	1.07 (0.16)	1.07 (0.13)	0.53
Rh4	Maternal	6.71 (1.32)	6.46 (1.32)	0.02
	Cord	6.81 (1.53)	6.43 (1.49)	0.002
	Cord/maternal	1.02 (0.17)	1.01 (0.15)	0.15
Rh5	Maternal	6.45 (1.05)	6.25 (0.97)	0.01
	Cord	6.52 (1.27)	6.34 (1.17)	0.07
	Cord/maternal	1.02 (0.16)	1.02 (0.15)	0.78

[a]The IPTp-SP group is the reference group for all p-values calculated

AMA1= Apical membrane antigen-1, DP= dihydroartemisinin-piperaquine, EBA=Erythrocyte-binding antigen, GLURP=Glutamate-rich protein, MSP1= Merozoite surface protein, Rh= reticulocyte-binding protein homologue, SP= sulfadoxine-pyrimethamine

Table 6.5 Association between cord blood IgG antibody levels and infant malaria during the first 12 months of life

IgG antibody	Categories of antibody in percentiles	Incidence rate ratio (95% CI)*	p-value	Time to first malaria episode, HR (95% CI)*	p-value
AMA1	≤25th percentile	reference group		reference group	
	>25-50th percentile	1.25 (0.98-1.59)	0.07	1.18 (0.88-1.59)	0.25
	>50th percentile	1.28 (1.03-1.59)	0.02	1.21 (0.94-1.57)	0.14
EBA140	≤25th percentile	reference group		reference group	
	>25-50th percentile	0.97 (0.77-1.24)	0.83	1.00 (0.75-1.34)	0.99
	>50th percentile	1.17 (0.95-1.45)	0.14	1.05 (0.82-1.35)	0.69
EBA175	≤25th percentile	reference group		reference group	
	>25-50th percentile	1.07 (0.83-1.37)	0.61	1.02 (0.76-1.36)	0.91
	>50th percentile	1.27 (1.03-1.57)	0.03	1.12 (0.87-1.44)	0.87
EBA181	≤25th percentile	reference group		reference group	
	>25-50th percentile	0.91 (0.72-1.16)	0.46	0.98 (0.73-1.31)	0.89
	>50th percentile	0.99 (0.80-1.22)	0.90	1.02 (0.79-1.32)	0.86
GLURP	≤25th percentile	reference group		reference group	
	>25-50th percentile	1.11 (0.86-1.42)	0.42	1.07 (0.79-1.44)	0.65
	>50th percentile	1.16 (0.94-1.44)	0.17	1.18 (0.91-1.54)	0.22
MSP1	≤25th percentile	reference group		reference group	
	>25-50th percentile	0.95 (0.75-1.21)	0.16	0.97 (0.73-1.30)	0.85
	>50th percentile	1.04 (0.85-1.29)	0.26	1.02 (0.79-1.31)	0.87
Rh2	≤25th percentile	reference group		reference group	
	>25-50th percentile	1.16 (0.92-1.47)	0.20	1.12 (0.84-1.50	0.43
	>50th percentile	1.03 (0.82-1.29)	0.79	1.02 (0.79-1.32)	0.87
Rh4	≤25th percentile	reference group		reference group	
	>25-50th percentile	0.94 (0.74-1.19)	0.62	0.89 (0.66-1.19)	0.42
	>50th percentile	0.94 (0.76-1.15)	0.55	0.93 (0.72-1.19)	0.56
Rh5	≤25th percentile	Reference group		reference group	
	>25-50th percentile	1.00 (0.78-1.29)	0.95	0.93 (0.69-1.25)	0.61
	>50th percentile	1.30 (1.06-1.60)	0.01	1.17 (0.91-1.51)	0.22

*Adjusted for maternal IPTp arm, maternal parasitaemia at enrolment, gravidity, and household type

AMA1= Apical membrane antigen-1, EBA=Erythrocyte-binding antigen, GLURP=Glutamate-rich protein, MSP1= Merozoite surface protein, Rh= reticulocyte-binding protein homologue

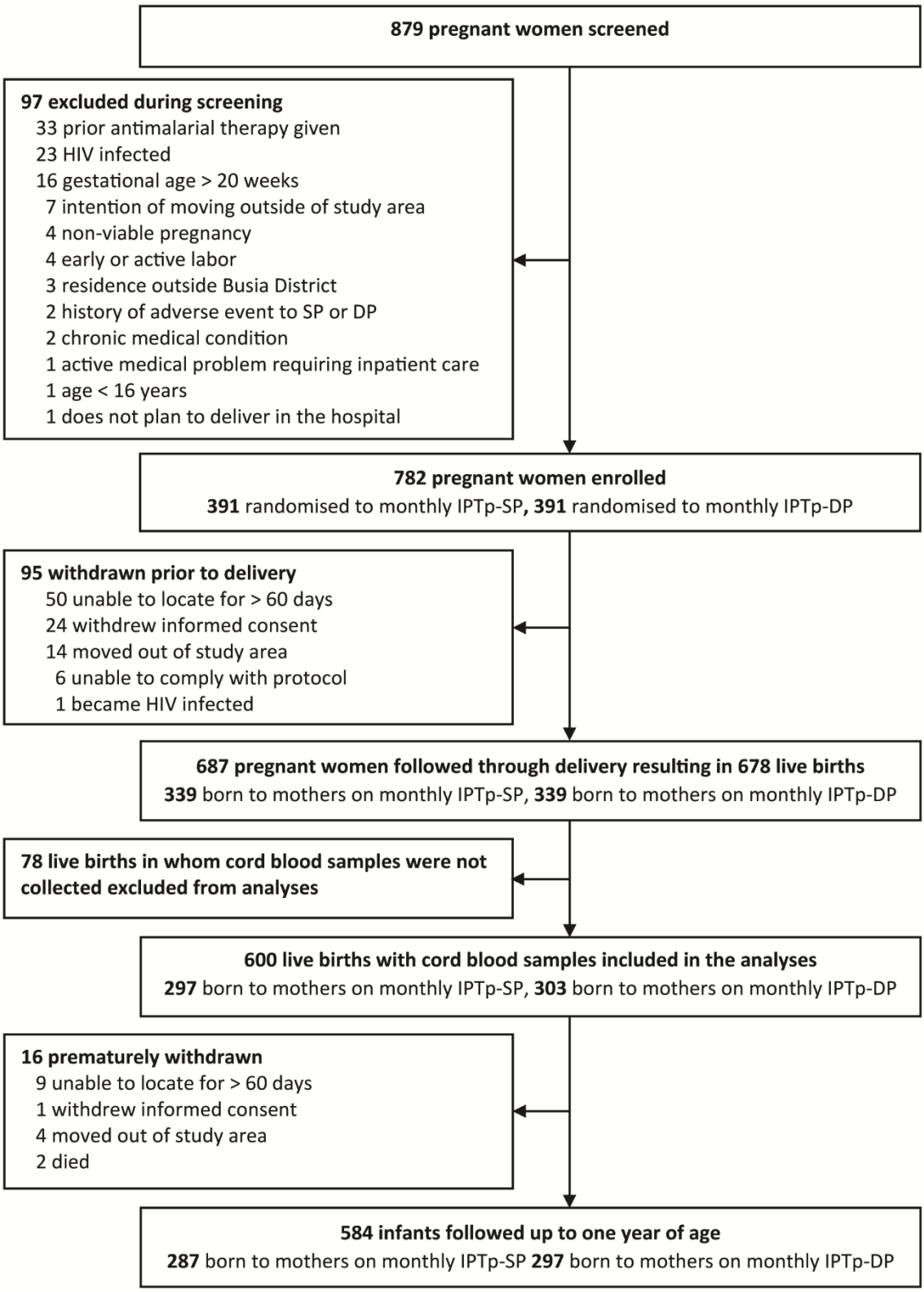

Figure 6.1 Study profile

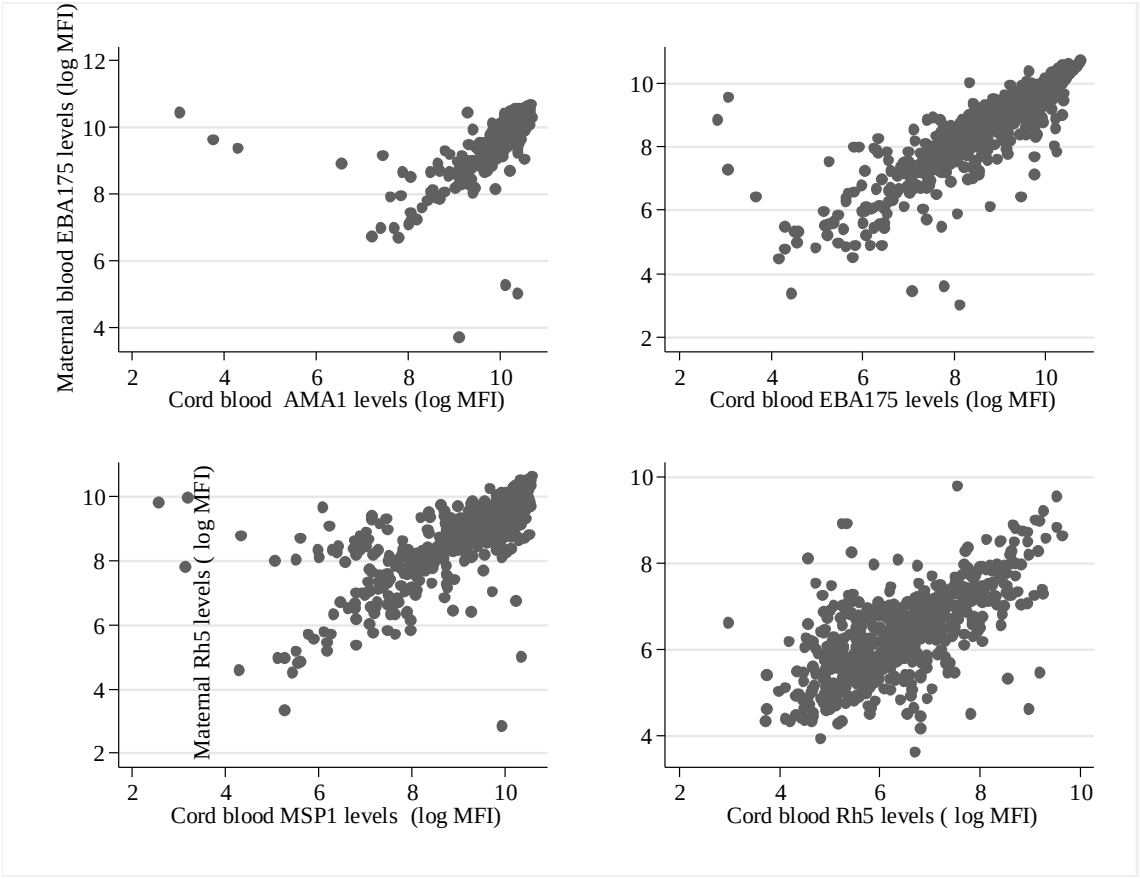

Figure 6.2 Correlations between maternal and cord blood IgG antibody levels for selected *P. falciparum antibodies*

AMA1= Apical membrane antigen-1, EBA175=Erythrocyte-binding antigen, MSP1= Merozoite surface protein-1, Rh5= reticulocyte-binding protein homologue-5

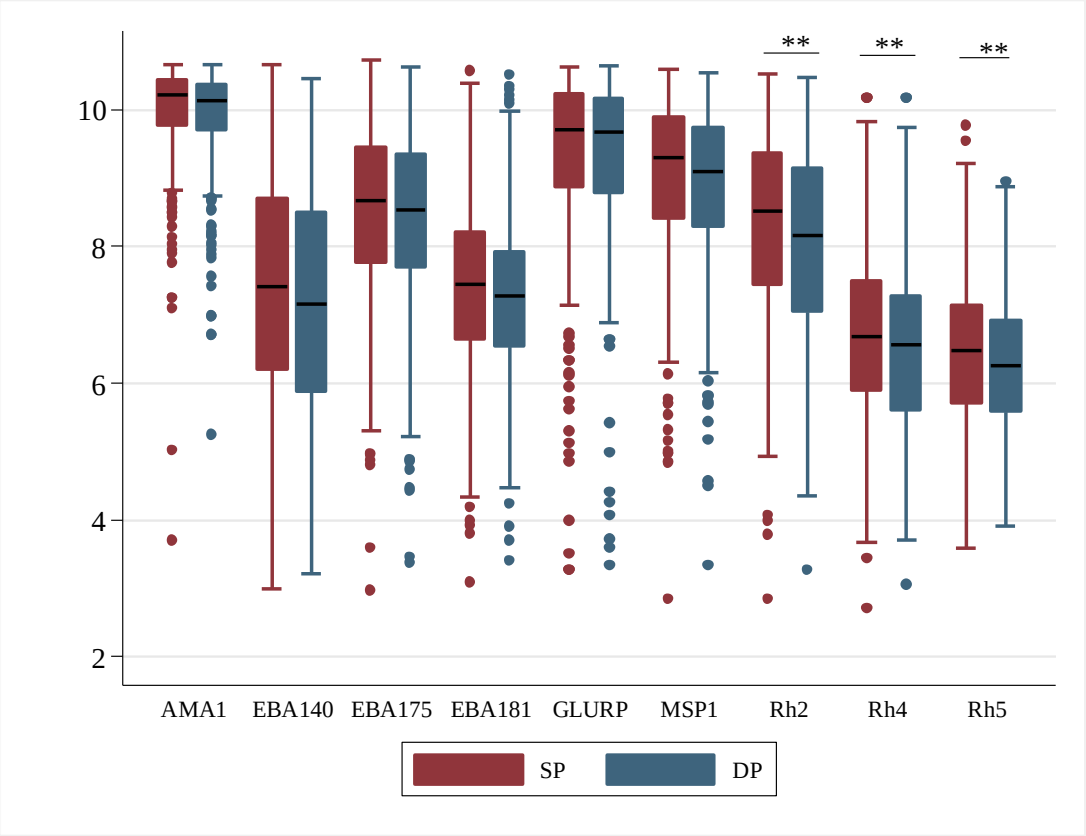

Figure 6.3 Maternal *P. falciparum* IgG antibody levels measured at delivery stratified IPTp arm

AMA1= Apical membrane antigen-1, AU= Arbitrary units, DP= dihydroartemisinin-piperaquine, EBA=Erythrocyte-binding antigen, GLURP=Glutamate-rich protein, IPTp= intermittent preventive treatment of malaria in pregnancy, MSP1= Merozoite surface protein-1, Rh= reticulocyte-binding protein homologue, SP= sulfadoxine-pyrimethamine
**P<0.05 but >0.006

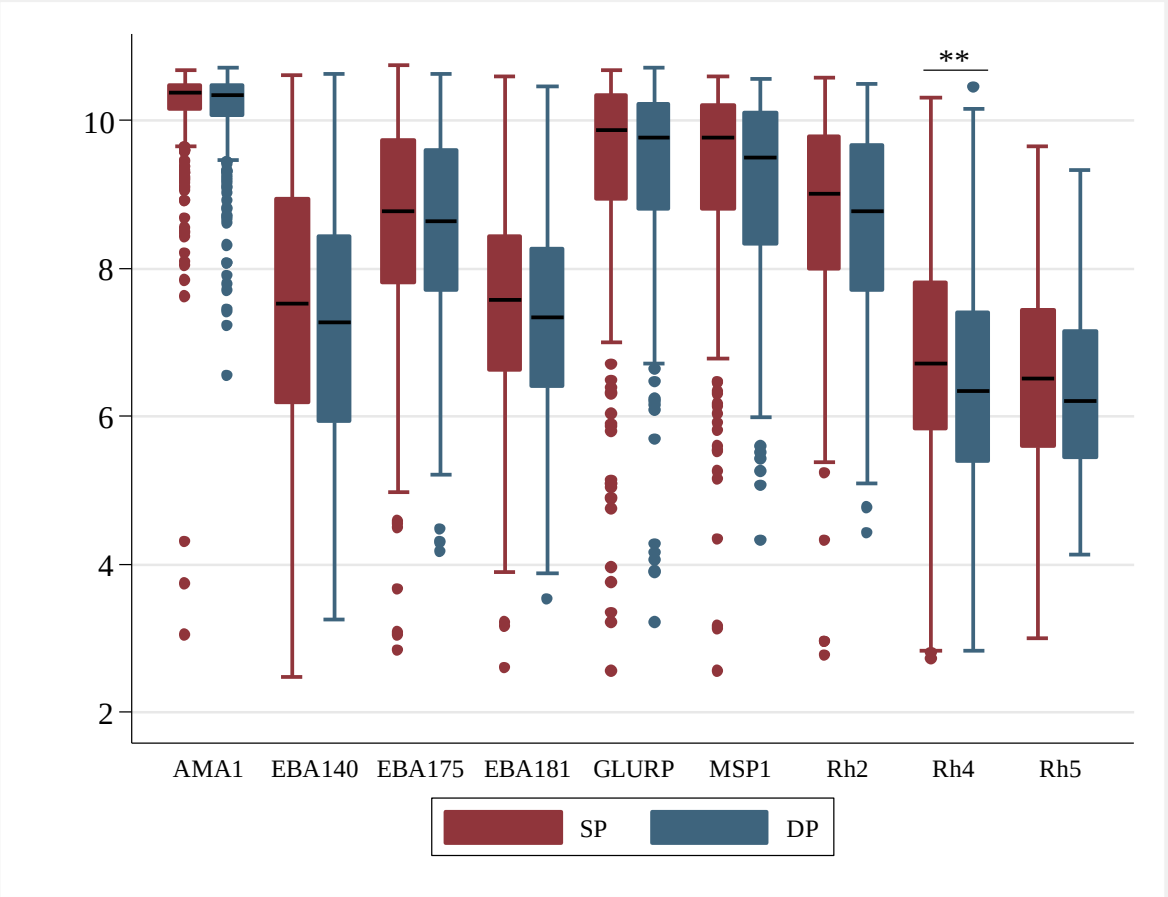

Figure 6.4 Cord blood *P. falciparum* IgG antibody levels measured at delivery stratified IPTp arm

AMA1= Apical membrane antigen-1, AU= Arbitrary units, DP= dihydroartemisinin-piperaquine, EBA=Erythrocyte binding antigen, GLURP=Glutamate-rich protein, IPTp= Intermittent preventive treatment of malaria in pregnancy, MSP1= Merozoite surface protein, Rh= reticulocyte-binding protein homologue, SP= sulfadoxine-pyrimethamine
**P<0.006

CHAPTER 7 DISCUSSION

7.1 Chapter introduction

This chapter summarises the main findings from this thesis, discusses their implications, highlights the study strengths and limitations, and provides an overall conclusion with recommendations for future research. This chapter has 8 sections. The first section summarises the study findings for each thesis objective; section 2 discusses the association between PM and the incidence of malaria infants; section 3 discusses association between IPTp or PM and secondary outcomes including infant mortality, non-malaria febrile illnesses, and anaemia; section 4 discusses the results of the impact of delivering IPTp DP vs SP to pregnant women on the incidence of malaria in their infants during the first 12 months of life; section 5 discusses the impact of IPTp and PM on *P. falciparum* IgG levels in umbilical cord blood; section 6 presents the thesis strength; section 7 addresses the limitations of the thesis; and section 8 provides the overall conclusion with recommendations for future research.

7.2 Summary of study findings

The systematic literature presented in chapter 2 highlighted that evidence on the association between MiP, or IPTp, and the risk of malaria in infants is lacking [1]. The majority of the studies included in the systematic literature review were observational studies, which suggested that MiP was associated with an increased risk of malaria during infancy. However, these studies were limited by potential confounding by malaria exposure which is shared by both the mother and the infant [2]. The best way to address this limitation would be to compare the risk of malaria in infants born to mothers randomised to different IPTp regimens where one of the regimens is more efficacious than the alternative regimen, however, few randomised controlled trials were included in the systematic literature review and these were limited by the failure to show a considerable difference in the risk of MiP between IPTp-regimens [3-6]. To address these limitations, this thesis used data collected from a birth cohort of infants born to HIV-uninfected pregnant women living in an area of high malaria transmission intensity, who participated in a double-blind randomised controlled trial of monthly IPTp with DP versus SP, to evaluate the impact of MiP on the risk of malaria in infancy. In this RCT, compared to IPTp-SP, IPTp-DP was associated with a 96% lower risk of malaria and 98% lower risk parasitaemia during pregnancy, and a 97%, 90%, and 54% lower risk of PM detected at delivery by microscopy, LAMP, or histology respectively [7]. The thesis had 3 main aims: 1) To compare the incidence of malaria during the first year of life among infants born to mothers with PM and those born mothers without PM; 2) To compare the incidence of malaria during the first year of life among infants

born to mothers randomised to IPTp with monthly DP and those born to mothers randomised to IPTp with monthly SP; and 3) To compare *P. falciparum* IgG antibody levels in cord blood of infants born to mothers who received monthly IPTp-DP vs those born to mothers who received monthly IPTp-SP.

The results of this thesis can be summarised in three key findings (Table 7.1): 1) Active PM and severe past PM were associated with an increased risk of malaria during infancy. However, active PM was only marginally associated with an increased risk of malaria in all infants, while severe past PM was associated with an increased risk of malaria, but only in male infants; 2) IPTp-DP was associated with a lower risk of malaria during infancy compared to IPTp-SP, but only in male infants; 3) There was no evidence of an association between PM or IPTp and infant mortality, non-malaria febrile illnesses or anaemia; 4) PM and IPTp were not associated with altered levels of *P. falciparum* IgG antibodies in cord blood. These key findings are discussed in detail in the next sections below.

Table 7.1 Summary of thesis objectives and main findings

Thesis objective	Key findings
Objective 1: To compare the incidence of malaria in infants during the first year of life among infants born to mothers with PM detected by microscopy, LAMP, or histology and those born to mothers without PM.	• Compared to infants born to mothers with no PM, infants born to mothers with active PM (parasites detected by microscopy, LAMP or histology) had a higher incidence of malaria during the first year of life, but the difference was of marginal significance • In the analysis stratified by infant sex, severe past PM (>20% HPF with malaria pigment deposition) was associated with a higher incidence of malaria, higher rate of first malaria episode, a higher incidence of complicated malaria and a higher prevalence of asymptomatic parasitaemia compared to no PM, but only in male infants • There was no evidence of an association between PM and incidence of all-cause hospitalisations, incidence of non-malaria febrile illnesses, and prevalence of anaemia • 89.7% of the effect of IPTp-DP vs SP on the incidence of malaria among male infants was mediated through prevention of PM
Objective 2: To compare the incidence of malaria during the first year of life in infants born to mothers who were randomised to receive monthly IPTp-DP versus monthly IPTp-SP.	• Overall IPTp-DP was associated with a 13% lower incidence of malaria in infants during the first year of life compared to IPTp-SP, but this difference was not statistically significant • IPTp DP was associated with a lower incidence of malaria and complicated malaria among male, but not female, infants • IPTp-DP was associated with a lower risk of hospitalisations, but only in male infants • IPTp-DP was not associated with the incidence of non-malaria febrile illnesses or prevalence of anaemia (Hb<10g/dL)
Objective 3: To compare *P. falciparum* antibody levels in cord blood of infants born to mothers who received monthly IPTp-DP vs those born to mothers who received monthly IPTp-SP.	• There was no association between PM and cord blood antibody levels or maternal-foetal transfer of *P. falciparum* IgG antibodies • There was no significant difference in cord blood levels of *P. falciparum* IgG antibodies among infants born to mothers who received IPTp-DP compared to those born to mothers who received IPTp-SP. IPTp-DP did not improve maternal-foetal transfer of *P. falciparum* IgG antibodies compared to IPTp-SP

7.3 Key finding 1: Placental malaria was associated with a higher incidence of malaria during infancy in male infants

We conducted a secondary analysis to assess for the association between PM and the incidence of malaria during infancy using data collected from a birth cohort of infants born to HIV-uninfected mothers who participated in the main trial, a double-blinded randomised controlled trial of monthly IPTp with DP versus SP. The incidence of malaria during the first 12 months of life was compared between infants born to mothers with active PM, mild to moderate past PM, or severe past PM, and infants born to mothers with no PM. In addition, mediation analysis was carried out to estimate what proportion of the observed association between IPTp-DP and the risk of malaria in infancy was mediated through the prevention of PM.

The results of the secondary analysis suggest that overall, active PM was associated with a higher incidence of malaria during infancy compared to no PM, although the difference was of borderline statistical significance, possibly due to the small sample size given that few infants were born to mothers who had active PM. An association between active PM and higher risk of malaria in infants has been previously reported in several studies in Uganda, Benin, Tanzania and Gabon [8-11]. However, most of these studies were observational studies and did not adjust for potential confounding by malaria exposure which is shared by the mother and her infant. Only one study, conducted in Benin, adjusted exposure to anopheles mosquitoes [9]. In this study PM detected by microscopy was associated with a higher risk of first parasitaemia during infancy, but only in infants who were resident in houses with insecticide-treated nets. In our study, we adjusted for malaria exposure using proxy measures of malaria exposure including maternal parasitaemia at enrolment, and household type (modern house with closed eaves vs traditional house built with mud and wattle with open eaves). Our study, together with previous studies suggest that active PM may be associated with a higher incidence of malaria in infancy, and that interventions that reduce the risk of active PM may be protective of malaria during infancy.

Importantly, we observed an association between severe past PM and the risk of malaria during infancy in male, but not female, infants. This association was consistent for all malaria outcomes including incidence of malaria, rate of first malaria episode, incidence of complicated malaria, and parasite prevalence. To our knowledge, this is the first study to report an association between severe past PM and the risk of malaria in male infants. Infants born to mothers with past PM have been previously reported to have a higher risk of malaria during the first year of life compared to infants born to mothers with no PM [12] although no effect modification by

infant sex has been previously reported. In our study, unlike severe past PM, mild-moderate past PM was not associated with a higher risk of malaria in male infants, suggesting that the severity of the past placental infection rather than the mere absence or presence of PM, may be more important. Our study findings suggest that in a setting of high malaria transmission intensity, the consequences of PM on the infant later in life may differ by sex and appear to be worse in male infants. Highly effective drugs for IPTp, which reduce the severity of PM, may be protective, but only in male infants.

The exact mechanisms through which PM may be acting to increase the risk of malaria during infancy are not well understood. However, reduced maternal-foetal transfer of antimalarial IgG antibodies and modulation of the foetal cell-mediated immune system following *in-utero* foetal exposure to malaria have been suggested as potential mechanisms. Previous studies have reported an association between PM and reduced maternal-foetal transfer of antimalarial IgG antibodies [13, 14]. However, there was no sex modification reported in this association. *In-utero* exposure to malaria antigens has been shown to be associated immune tolerance which may affect infant immunity to infectious diseases including malaria [15-17]. Furthermore, male infants exposed to malaria including PM *in-utero* have been reported to have a higher proportion of regulatory T-cells in cord blood compared female infants with similar exposure [18] suggesting that in-utero malaria exposure may result in immune tolerance in male but not female infants. The findings of this thesis add to the growing literature of sex-disparities in risk of adverse birth outcomes and infectious diseases suggesting that male infants are at a higher risk [19-21].

The observed association between severe past PM and the risk of malaria supports the hypothesis that IPTp with highly efficacious drugs, which may reduce the severity of PM, particularly in high malaria transmission settings where pregnant women are at risk of recurrent *P. falciparum* infections, may be protective against malaria during infancy in male infants. This is further supported by the results of the mediation analysis which suggested to that a large proportion (89.7%) of the protective effect of IPTp-DP, compared IPTp-SP, against malaria during infancy was mediated through prevention of PM among male infants, though this was not statistically significant.

7.4 Key finding 2: IPTp-DP is associated with a lower risk of malaria, complicated malaria, and all-cause hospitalisations in male infants

Recent studies have shown that DP is a highly effective and promising alternative to SP for IPTp. Compared to IPTp-SP, IPTp-DP has been found to reduce the risk of clinical malaria and

parasitaemia during pregnancy, and reduce the risk of PM at delivery; but no clear difference in the risk of adverse birth outcomes has been shown [7, 22, 23]. Several observational studies have reported an association between MiP and malaria in infancy suggesting that highly effective interventions, such as IPTp-DP which reduce the burden of MiP, including PM, may protect the infant against malaria in early life. This may have implications on the choice of alternative drugs to replace SP for IPTp. To confirm this observation, the incidence of malaria during infancy was compared among infants born to mothers randomised to monthly IPTp with DP vs SP.

This is the first double-blind, randomised, controlled trial to compare the incidence of malaria in infants born to mothers randomised to monthly IPTp-DP compared to those born to mothers randomised to monthly IPTp-SP. The second hypothesis was that infants born to mothers randomised to monthly IPTp-DP would have a lower incidence of malaria during the first year of life compared to those born to mothers randomised to monthly IPTp-SP. The results of this analysis presented in chapter 5 suggest that monthly IPTp-DP is associated with a lower incidence of malaria during infancy, compared to monthly IPTp-SP [24]. However, this difference was not statistically significant, possibly due to a lower-than-expected incidence of malaria in infants born to mothers in the control arm (IPTp-SP). To my knowledge, this is the first study to show that IPTp with highly effective drugs may be associated with a lower incidence of malaria in infants compared to the standard of care. Previous studies that compared the incidence of malaria in infants born to mothers randomised to different IPTp regimens found no evidence of a difference in malaria in infants. There was no difference in the incidence of malaria in infants born to mothers randomised to IPTp with MQ compared to those born to mothers who received IPTp-SP in Benin, Gabon, Tanzania, and Mozambique [25], in infants born to mothers randomised to IPTp-SP compared to placebo in Mozambique [12], in infants born to mothers randomised to intermittent screening and treatment with AL compared to those born to mothers who received IPTp-SP in Ghana [26], or in infants born to mothers randomised to monthly community-scheduled screening and treatment of malaria during pregnancy plus IPTp-SP (CSST/IPTP-SP) compared to IPTp-SP in Burkina Faso [27]. These studies were limited by the failure to show a substantial difference between the chemoprevention interventions on the risk of MiP [3-6].

Interestingly, the association between IPTp regimens and the incidence of malaria was modified by infant sex, such that the reduction in the incidence of malaria associated with IPTp-DP, as compared to IPTp-SP, was only observed in male infants. Consistent with this observation, the incidence of complicated malaria was also lower in male infants born to mothers who received IPTp-DP compared to those born mothers who received IPTp-SP. In support of these findings, a

previous study reported a lower incidence of malaria during the first two years of life, in male children receiving chemoprevention with DP born to mothers randomised to monthly IPTp-DP versus IPTp-SP given every two months [28]. However, this difference was not statistically significant, possibly due to a limited sample size. This difference in the effect of IPTp-DP on the incidence of malaria among male and female infants could be explained by the effect of severe past PM, which in chapter 4, was reported to be associated with a higher incidence of malaria and complicated malaria in male but not female infants. IPTp-DP may be acting by reducing the risk of severe past PM, which may have negative consequences in male but not female infants.

Similarly, the association between IPTp and the incidence of all-cause hospitalisations was also modified by infant sex. The rate of all-cause hospitalisations was lower in male infants born to mothers received IPTp-DP than in male infants born to mothers who received IPTp-SP, but no difference was observed among female infants [24]. This could possibly be partly be explained by the observed lower incidence of complicated malaria among male infants born to mothers who received IPTp-DP compared to male infants born to mothers who received IPTp-SP. All together, these findings suggest that compared to IPTp-SP, IPTp-DP may be protective against malaria and hospitalisations in male infants.

7.5 Key finding 3: No evidence of an association between IPTp or PM and infant mortality, non-malaria febrile illnesses, and anaemia

Previous studies have reported increased mortality in infants born to mothers with PM [12, 29] and that prevention of malaria in pregnancy may improve infant survival [30]. However, in this study there was no strong evidence of improved survival in infants born to mothers receiving IPTp-DP compared to those born to mothers receiving IPTp-SP, although the infant mortality was lower (but not significantly so) among infants born to mothers on IPTp-DP. The reasons for the lack of association in this study could be due to a small number of deaths observed during follow-up possibly due to close follow-up and access to better health care, which resulted in type II error.

No associations between IPTp-DP or PM and the risk of non-malaria febrile illnesses during infancy were observed in this study, suggesting that effective prevention of MiP may have no effect on the risk of non-malaria illnesses. In contrast to these findings, previous studies conducted in Burkina Faso [27] and Benin [31] reported an association between prevention of MiP and PM, and the risk of non-malaria febrile illnesses in infants. Prevention of MiP with community-based screening and testing, in addition to IPTp-SP was found to be protective against non-malaria febrile illnesses among infants in Burkina Faso during the first year of life

compared to IPTp-SP alone [27]. However, no association was observed between MiP and the risk of non-malaria febrile illnesses in infants in that study, contrary to the reported association between PM and a higher risk of non-malaria febrile illnesses during infancy reported by Rachas et al in Benin [31].

Moreover, there was no association between IPTp or PM and the risk of anaemia during infancy, in this study, which is in contrast to a previous study in Benin, which reported a higher risk of anaemia during infancy among infants born to mothers with PM than in those born to mothers without PM [32]. In agreement with our study findings, there was no association between IPTp or PM and the risk of anaemia in infants in a study conducted in Ghana [26]. Together, these data suggest that IPTp and PM may have a greater impact on malaria specific outcomes than non-malaria outcomes in infants.

7.6 Key finding 4: Placental malaria and IPTp were not associated with levels of *P. falciparum* IgG antibodies and both did not affect maternal-foetal transfer of IgG antibodies.

In this study, in chapter 4, we observed that infants born to mothers with severe past PM had a higher risk of malaria during the first 12 months of life compared to infants born to mothers with no PM. This association was observed only in males. We also observed that male infants born to mothers who received monthly IPTp DP had a lower risk of malaria compared to those born to mothers who received monthly IPTp-SP [24]. Using maternal and cord blood samples collected at delivery from HIV-uninfected pregnant women who had taken part in a randomised controlled trial, we assessed whether PM and IPTp may affect *P. falciparum* IgG antibody levels in cord blood or maternal-foetal transfer of *P. falciparum* IgG antibodies.

Previous studies reported that PM is associated with a reduced maternal-foetal of IgG antibodies to *P. falciparum* which may affect the newborn's immunity to *P. falciparum* [13, 14]. Contrary to findings from previous studies, there was no evidence that PM is associated with reduced cord blood levels or maternal-foetal transfer of *P. falciparum* IgG antibodies to AMA1, EBA140, EBA175, EBA181, GLURP, MSP1, Rh2, Rh4, and Rh5. Our findings suggest that in a setting of high malaria transmission intensity, PM may not affect the transfer of these measured *P. falciparum* IgG antibodies from the mother to the foetus. The reason for the observed differences in the findings of this study and previous studies could be due to differences in study design, and malaria transmission intensity setting. In our study, women were randomised to receive monthly IPTp-SP vs DP, while the study conducted in Mozambique was a randomised trial of 2 dose IPTp SP vs placebo [5, 13]. Also, while the study in Benin was conducted in an area of moderate

malaria transmission intensity [13], our study was conducted in a very high transmission intensity where most of the adult residents in this area usually have high levels of circulating antibodies to *P. falciparum* antigens and therefore pregnant women will be more likely to transfer antibodies to the foetus.

IPTp-DP has been previously shown to reduce the burden malaria during pregnancy including reducing the risk of PM during pregnancy compared to IPTp-SP [7, 22, 23] suggesting that IPTp-DP may improve the maternal-foetal transfer of IgG antibodies to *P. falciparum* antigens. In this study, maternal-foetal transfer was similar among infants born to mothers who received IPTp-DP compared to infants born to mothers who received IPTp-SP suggesting that effective IPTp may not improve maternal-foetal transfer of *P. falciparum* IgG antibodies. This is possibly because effective IPTp may reduce the level of maternal *P. falciparum* IgG antibodies [33] due to reduced exposure to blood-stage parasite forms which reduces the quantity of antibodies transferred such that the increase of maternal-foetal transfer of *P. falciparum* IgG due to the reduced risk of PM is cancelled out by a decrease in the level of the maternal antibodies available for transfer to the foetus. Nevertheless, in this study, levels of IgG antibodies in maternal blood at delivery were similar among mothers who received IPTp-DP compared to mothers who received IPTp-SP.

It is widely believed that maternal antibodies transferred to the foetus protect the newborn against malaria during the first months of life [34]. However, in this study, like previous studies conducted in Ghana and Burkina Faso [35, 36], higher levels of IgG antibodies to *P. falciparum* antigens were not associated with protection against malaria during the first 12 months of life. Instead, higher levels of some *P. falciparum* IgG antibodies were associated with a trend towards a higher incidence of malaria during the first year of life suggesting that higher levels of *P. falciparum* IgG antibodies in cord blood may be an indicator of malaria exposure [36].

Together, these results suggest that in an area of high malaria transmission intensity PM does not affect maternal-foetal transfer of *P. falciparum* IgG antibodies and effective IPTp with DP may not increase maternal to foetal transfer of antibodies.

7.7 Implication of the study findings

Overall, our study findings show that in an area of high malaria transmission intensity, the severity of PM, detected by the quantity of malaria pigment deposited in placental fibrin, rather than the mere presence or absence of PM that, was associated with a higher incidence of malaria during infancy, but only in male infants. This suggests that interventions which reduce the

severity of PM may be protective against malaria in male infants resident in a high malaria transmission intensity setting.

The results of the comparison of incidence of malaria during infancy among infants born to mothers who were randomised to receive monthly IPTp with DP vs those born to mothers randomised to receive monthly IPTp-SP suggested that IPTp was associated with a moderate protective effect against malaria during the first 12 months of life among male infants [24]. This could be due largely to its impact on reducing the severity of past PM which was shown to be associated with a higher risk of malaria in male infants. This is supported by results of the mediation analysis which showed that a greater proportion of the effect of IPTp-DP versus IPTp-SP on the incidence of malaria in male infants was mediated through reduction of PM.

In this study, the observed association between PM, IPTp and the incidence of malaria could not be explained by the suggested reduced maternal-foetal transfer of *P. falciparum* IgG antibodies which was reported in previous studies [13, 14]. Both PM and IPTp did not impact on the maternal-foetal transfer of specific *P. falciparum* IgG antibodies and therefore did not impact on the level of these antibodies in cord blood. This suggests that the effect of PM on other factors such as cell-mediated immunity [37, 38] and IgG antibody acquisition during infancy [39], may explain the observed reduced incidence of malaria among male infants born to mothers with severe past PM.

Altogether, the findings of this thesis suggest that in a setting of high malaria transmission intensity, where pregnant women are more likely to get multiple infections, severe PM is associated with a higher risk of malaria in male infants and that IPTp with monthly DP, a highly effective drug compared to SP, which reduces the severity of PM may be protective against malaria in male infants during the first year of life.

7.8 Thesis strengths

This study had several strengths including a rigorous study design, and diagnosis and classification of PM. The randomised, blinded design of this study reduced the potential effect of confounding factors and other biases which would have been difficult to adjust for during analysis. The substantial differences in efficacy between IPTp-DP and IPTp-SP on the risk of MiP, including PM, made it possible to test the hypothesis that highly effective interventions which substantially reduce the risk of MiP are protective against malaria during infancy. In addition, the birth cohort nature of the study, with monthly follow-up of infants at a dedicated study clinic, allowed for assessment of both asymptomatic parasitaemia and clinical malaria right from birth to 12 months of age.

Diagnosing PM has been a challenge for previous studies that assessed associations between PM and malaria risk in infancy [1]. To address this, we used a combination of different methods to diagnose PM. In this thesis, PM was diagnosed from placental blood by microscopy and LAMP, and from placental tissue by histology. The advantage of using microscopy is that it is inexpensive and relatively easy to use, however, it only detects active or chronic infection and has limited sensitivity for detecting PM compared to molecular methods like LAMP, and histology [40]. In this study, 9% of the mothers had PM detected by microscopy, compared to 22% which were detected by LAMP [7]. Like microscopy for detecting PM, LAMP detects active, or chronic infections, but not past infections. Placental histology is more sensitive than both microscopy and LAMP; in this study over 60% of the mothers had PM detected by histology compared to only 22% by LAMP [7]. The main limitation for histology is that it requires advanced technical skills which are not widely available.

Leveraging on the different methods for detection of PM, this thesis used a modified system for classifying PM based on Muehlenbachs et al [41] and Ismail et al [42] which included four categories of PM: 1) no PM, 2) active PM, 3) mild-moderate past PM, and 4) severe past PM. This allowed us to evaluate severity of PM as shown by the quantity of malaria pigment deposition which has been shown to be associated with adverse birth outcomes [43], and in this study, was found to be associated with a higher incidence malaria in male infants. Future studies evaluating the association between PM and infant outcomes in high malaria transmission intensity settings should consider including PM categories based on severity of past infection.

7.9 Thesis limitations

This study also had several limitations which have been briefly discussed in the results chapters. Here, the limitations are discussed in detail. First, IPTp is recommended in pregnant women in both moderate and high malaria transmission settings [44]. However, this study was conducted in a setting of very high malaria transmission intensity, which limited the generalisation of the thesis findings to settings of moderate malaria transmission intensity.

Second, the study had a limited sample size which reduced power to detect a significant difference, if one truly existed. It was expected that the incidence of malaria during infancy among infants born to mothers who received IPTp-SP would be at least 3 episodes per person year. However, in this study the incidence of malaria among infants born to mothers who received IPTp-SP was approximately 2 episodes per person year, which reduced the power of the study. Furthermore, stratified analyses, including those stratifying for infant sex and age, although planned *a priori*, were not put into consideration at study inception. This too limited the power of the study to detect significant differences within strata if true differences existed.

Third, in this study, administration of all doses of IPTp-SP was directly observed while, for IPTp-DP, only the 1st daily doses of the three daily doses given monthly, were administered directly observed. This could have limited the adherence of IPTp-DP if some mothers did not take their 2nd or 3rd daily doses leading to under estimation of the effect of IPTp-DP on the risk of malaria in infancy compared to IPTp-SP. However, the approach of administering IPTp-DP with partial directly observed therapy is what would be done in routine care settings as administration of all three daily doses by direct observation would be very challenging.

Fourth, in the assessment of the association between PM and the incidence of malaria in infancy, it is important to measure malaria transmission intensity at the level of the household so that this potential confounding by malaria exposure shared by both the mother and her infant can be adjusted for. However, we were unable to directly measure malaria transmission intensity using entomological measures (such as entomological inoculation rate) and epidemiological measures (such as parasite prevalence) due to resource limitations. Nonetheless, maternal parasitaemia detected at enrolment by microscopy and qPCR, and household type (whether modern house with nets in ventilators or traditional house with open eaves) were considered as proxy measures of malaria transmission intensity and these were adjusted for in the analysis.

Fifth, in this study, placental inflammation was not included the grading of PM. The extent of placental inflammation has been shown to be associated with adverse birth outcomes similar to the level of malaria pigment deposition [41], but there is currently no data on the effect of placental inflammation on the incidence of malaria during infancy. Future studies should consider evaluating the effect of placental inflammation on the incidence of malaria during infancy.

Finally, the mediation analysis performed for this study was limited by confounding. In this analysis, it was assumed that all confounding factors in the association between IPTp and the risk of PM, and between PM and the incidence of malaria were measured or controlled for. This assumption is valid for the association between IPTp and the risk of PM because the IPTp interventions were randomised which allowed control of both known and unknown confounders. However, in the association between PM and the incidence of malaria in infants only maternal parasitaemia status at enrolment, gravidity, house-hold type, and maternal IPTp arm were adjusted for. It is possible that there are unmeasured confounders which were not adjusted for.

7.10 Conclusion

Overall, this thesis found that in a setting of high malaria transmission intensity, severe past PM was associated with a higher incidence of malaria, complicated malaria, and parasitaemia in male infants. Monthly IPTp-DP, which has been shown to significantly reduce the risk of malaria, parasitaemia and PM during pregnancy compared to monthly IPTp-SP [7, 22, 23], was associated with a lower incidence of malaria, and complicated malaria, during infancy, but only among male infants. This suggests that IPTp with highly effective drugs could be protective against malaria in infants. Future IPTp studies conducted in both settings of moderate and high malaria transmission intensity, should consider follow-up of infants to evaluate the benefits of IPTp to the infant beyond the neonatal period, and assess the role of infant sex. Future studies in high malaria transmission settings evaluating the association between PM and infant outcomes should consider the severity of PM based on the proportion of HPF with malaria pigment deposition in fibrin.

7.12 References

1. Kakuru A, Staedke SG, Dorsey G, Rogerson S, Chandramohan D: **Impact of *Plasmodium falciparum* malaria and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants: a systematic review.** *Malar J* 2019, **18:**304.

2. Cairns M, Gosling R, Chandramohan D: **Placental malaria increases malaria risk in the first 30 months of life: not causal.** *Clin Infect Dis* 2009, **48:**497-498; author reply 498-499.

3. Consortium C: **Community-based malaria screening and treatment for pregnant women receiving standard intermittent preventive treatment with sulfadoxine-pyrimethamine: a multicenter (The Gambia, Burkina Faso, and Benin) cluster-randomized controlled trial.** *Clin Infect Dis* 2018, **68:**586-596.

4. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, Kakolwa MA, Abdulla S, Accrombessi M, Aponte JJ, Akerey-Diop D, Basra A, Briand V, et al: **Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-negative women: a multicentre randomized controlled trial.** *PLoS Med* 2014, **11:**e1001733.

5. Menendez C, Bardaji A, Sigauque B, Romagosa C, Sanz S, Serra-Casas E, Macete E, Berenguera A, David C, Dobano C, et al: **A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic.** *PLoS One* 2008, **3:**e1934.

6. Tagbor H, Cairns M, Bojang K, Coulibaly SO, Kayentao K, Williams J, Abubakar I, Akor F, Mohammed K, Bationo R, et al: **A non-inferiority, individually randomized trial of intermittent screening and treatment versus intermittent preventive treatment in the control of malaria in pregnancy.** *PLoS One* 2015, **10:**e0132247.

7. Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, Ruel T, Opira B, Ochokoru H, Ategeka J, Nayebare P, et al: **Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in pregnancy: a double-blind, randomised, controlled, superiority trial.** *Lancet* 2019, **393:**1428-1439.

8. De Beaudrap P, Turyakira E, Nabasumba C, Tumwebaze B, Piola P, Boum Ii Y, McGready R: **Timing of malaria in pregnancy and impact on infant growth and morbidity: a cohort study in Uganda.** *Malar J* 2016, **15:**92.

9. Le Port A, Watier L, Cottrell G, Ouedraogo S, Dechavanne C, Pierrat C, Rachas A, Bouscaillou J, Bouraima A, Massougbodji A, et al: **Infections in infants during the first 12**

months of life: role of placental malaria and environmental factors. *PLoS ONE [Electronic Resource]* 2011, **6:**e27516.

10. Mutabingwa TK, Bolla MC, Li JL, Domingo GJ, Li X, Fried M, Duffy PE: **Maternal malaria and gravidity interact to modify infant susceptibility to malaria.** *PLoS Med* 2005, **2:**e407.

11. Schwarz NG, Adegnika AA, Breitling LP, Gabor J, Agnandji ST, Newman RD, Lell B, Issifou S, Yazdanbakhsh M, Luty AJ, et al: **Placental malaria increases malaria risk in the first 30 months of life.** *Clin Infect Dis* 2008, **47:**1017-1025.

12. Bardaji A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, Mabunda S, Alonso PL, Menendez C: **Impact of malaria at the end of pregnancy on infant mortality and morbidity.** *J Infect Dis* 2011, **203:**691-699.

13. Dechavanne C, Cottrell G, Garcia A, Migot-Nabias F: **Placental malaria: decreased transfer of maternal antibodies directed to *Plasmodium falciparum* and impact on the incidence of febrile infections in infants.** *PLoS ONE [Electronic Resource]* 2015, **10:**e0145464.

14. Moro L, Bardaji A, Nhampossa T, Mandomando I, Serra-Casas E, Sigauque B, Cistero P, Chauhan VS, Chitnis CE, Ordi J, et al: **Malaria and HIV infection in Mozambican pregnant women are associated with reduced transfer of antimalarial antibodies to their newborns.** *J Infect Dis* 2015, **211:**1004-1014.

15. Engelmann I, Santamaria A, Kremsner PG, Luty AJ: **Activation status of cord blood gamma delta T cells reflects in utero exposure to *Plasmodium falciparum* antigen.** *J Infect Dis* 2005, **191:**1612-1622.

16. Fievet N, Varani S, Ibitokou S, Briand V, Louis S, Perrin RX, Massougbogji A, Hosmalin A, Troye-Blomberg M, Deloron P: ***Plasmodium falciparum* exposure in utero, maternal age and parity influence the innate activation of foetal antigen presenting cells.** *Malar J* 2009, **8:**251.

17. Gbedande K, Varani S, Ibitokou S, Houngbegnon P, Borgella S, Nouatin O, Ezinmegnon S, Adeothy AL, Cottrell G, Massougbodji A, et al: **Malaria modifies neonatal and early-life toll-like receptor cytokine responses.** *Infect Immun* 2013, **81:**2686-2696.

18. Prahl M, Jagannathan P, McIntyre TI, Auma A, Wamala S, Nalubega M, Musinguzi K, Naluwu K, Sikyoma E, Budker R, et al: **Sex disparity in cord blood FoxP3(+) CD4 T regulatory cells in infants exposed to malaria in utero.** *Open Forum Infect Dis* 2017, **4:**ofx022.

19. Klein SL, Flanagan KL: **Sex differences in immune responses.** *Nature Reviews Immunology* 2016, **16:**626.

20. Mondal D, Galloway TS, Bailey TC, Mathews F: **Elevated risk of stillbirth in males: systematic review and meta-analysis of more than 30 million births.** *BMC Med* 2014, **12:**220.

21. Muenchhoff M, Goulder PJ: **Sex differences in pediatric infectious diseases.** *J Infect Dis* 2014, **209 Suppl 3:**S120-126.

22. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, Ouma P, Were V, Laserson K, Katana A, et al: **Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial.** *Lancet* 2015, **386:**2507-2519.

23. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, Opira B, Olwoch P, Ategeka J, Nayebare P, et al: **Dihydroartemisinin-piperaquine for the prevention of malaria in pregnancy.** *N Engl J Med* 2016, **374:**928-939.

24. Kakuru A, Jagannathan P, Kajubi R, Ochieng T, Ochokoru H, Nakalembe M, Clark TD, Ruel T, Staedke SG, Chandramohan D, et al: **Impact of intermittent preventive treatment of malaria in pregnancy with dihydroartemisinin-piperaquine versus sulfadoxine-pyrimethamine on the incidence of malaria in infancy: a randomized controlled trial.** *BMC Med* 2020, **18:**207.

25. Ruperez M, Gonzalez R, Mombo-Ngoma G, Kabanywanyi AM, Sevene E, Ouedraogo S, Kakolwa MA, Vala A, Accrombessi M, Briand V, et al: **Mortality, morbidity, and developmental outcomes in infants born to women who received either mefloquine or sulfadoxine-pyrimethamine as intermittent preventive treatment of malaria in pregnancy: a cohort study.** *PLoS Medicine / Public Library of Science* 2016, **13:**e1001964.

26. Awine T, Belko MM, Oduro AR, Oyakhirome S, Tagbor H, Chandramohan D, Milligan P, Cairns M, Greenwood B, Williams JE: **The risk of malaria in Ghanaian infants born to women managed in pregnancy with intermittent screening and treatment for malaria or intermittent preventive treatment with sulfadoxine/pyrimethamine.** *Malar J* 2016, **15:**46.

27. Natama HM, Rovira-Vallbona E, Sorgho H, Some MA, Traore-Coulibaly M, Scott S, Zango SH, Sawadogo O, Zongo SC, Valea I, et al: **Additional screening and treatment of malaria during pregnancy provides further protection against malaria and non-malarial fevers during the first year of life.** *J Infect Dis* 2018, **217:**1967-1976.

28. Jagannathan P, Kakuru A, Okiring J, Muhindo MK, Natureeba P, Nakalembe M, Opira B, Olwoch P, Nankya F, Ssewanyana I, et al: **Dihydroartemisinin-piperaquine for intermittent**

preventive treatment of malaria during pregnancy and risk of malaria in early childhood: a randomized controlled trial. *PLoS Med* 2018, **15:**e1002606.

29. Guyatt HL, Snow RW: **Malaria in pregnancy as an indirect cause of infant mortality in sub-Saharan Africa.** *Trans R Soc Trop Med Hyg* 2001, **95:**569-576.

30. Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, Bennett A, Hutchinson P, Steketee RW: **Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa.** *Lancet Infect Dis* 2012, **12:**942-949.

31. Rachas A, Le Port A, Cottrell G, Guerra J, Choudat I, Bouscaillou J, Massougbodji A, Garcia A: **Placental malaria is associated with increased risk of nonmalaria infection during the first 18 months of life in a Beninese population.** *Clin Infect Dis* 2012, **55:**672-678.

32. Accrombessi M, Ouedraogo S, Agbota GC, Gonzalez R, Massougbodji A, Menendez C, Cot M: **Malaria in pregnancy Is a predictor of infant haemoglobin concentrations during the first year of life in Benin, West Africa.** *PLoS One* 2015, **10:**e0129510.

33. Stephens JK, Kyei-Baafour E, Dickson EK, Ofori JK, Ofori MF, Wilson ML, Quakyi IA, Akanmori BD: **Effect of IPTp on *Plasmodium falciparum* antibody levels among pregnant women and their babies in a sub-urban coastal area in Ghana.** *Malar J* 2017, **16:**224.

34. Akum AE, Minang JT, Kuoh AJ, Ahmadou MJ, Troye-Blomberg M: ***Plasmodium falciparum* inhibitory capacities of paired maternal-cord sera from south-west province, Cameroon.** *J Trop Pediatr* 2005, **51:**182-190.

35. Riley EM, Wagner GE, Ofori MF, Wheeler JG, Akanmori BD, Tetteh K, McGuinness D, Bennett S, Nkrumah FK, Anders RF, Koram KA: **Lack of association between maternal antibody and protection of African infants from malaria infection.** *Infect Immun* 2000, **68:**5856-5863.

36. Kangoye DT, Nebie I, Yaro JB, Debe S, Traore S, Ouedraogo O, Sanou G, Soulama I, Diarra A, Tiono A, et al: ***Plasmodium falciparum* malaria in children aged 0-2 years: the role of foetal haemoglobin and maternal antibodies to two asexual malaria vaccine candidates (MSP3 and GLURP).** *PLoS One* 2014, **9:**e107965.

37. Harrington WE, Kakuru A, Jagannathan P: **Malaria in pregnancy shapes the development of foetal and infant immunity.** *Parasite Immunol* 2019, **41:**e12573.

38. Brustoski K, Moller U, Kramer M, Hartgers FC, Kremsner PG, Krzych U, Luty AJ: **Reduced cord blood immune effector-cell responsiveness mediated by CD4+ cells induced in utero as a consequence of placental *Plasmodium falciparum* infection.** *J Infect Dis* 2006, **193:**146-154.

39. Bonner PC, Zhou Z, Mirel LB, Ayisi JG, Shi YP, van Eijk AM, Otieno JA, Nahlen BL, Steketee RW, Udhayakumar V: **Placental malaria diminishes development of antibody responses to *Plasmodium falciparum* epitopes in infants residing in an area of western Kenya where *P. falciparum* is endemic.** *Clin Diagn Lab Immunol* 2005, **12:**375-379.

40. Rogerson SJ, Mkundika P, Kanjala MK: **Diagnosis of *Plasmodium falciparum* malaria at delivery: comparison of blood film preparation methods and of blood films with histology.** *J Clin Microbiol* 2003, **41:**1370-1374.

41. Muehlenbachs A, Fried M, McGready R, Harrington Whitney E, Mutabingwa Theonest K, Nosten F, Duffy Patrick E: **A novel histological grading scheme for placental malaria applied in areas of high and low malaria transmission.** *The Journal of Infectious Diseases* 2010, **202:**1608-1616.

42. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, Hirt R, Cardesa A, Alonso PL: **Placental pathology in malaria: a histological, immunohistochemical, and quantitative study.** *Hum Pathol* 2000, **31:**85-93.

43. Ategeka J, Kakuru A, Kajubi R, Wasswa R, Ochokoru H, Arinaitwe E, Adoke Y, Jagannathan P, R. Kamya M, Muehlenbachs A, et al: **Relationships between measures of malaria at delivery and adverse birth outcomes in a high-transmission area of Uganda.** *The Journal of Infectious Diseases* 2020.

44. World Health Organization: **WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP).** [http://www.who.int/malaria/publications/atoz/iptp-sp-updated-policy-brief-24jan2014.pdf]

LIST OF APPENDICES

Appendix A: Admissions offer

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

OFFER OF ADMISSION

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Applicant Name:	**Abel KAKURU**		
Address:	**INFECTIOUS DISEASES RESEARCH COLLABORTION, TORORO DISTRICT HOSPITAL, TORORO, UG**		
Email Address:	**abelkakuru@gmail.com**		
Course:	**Res - MPhil/PhD – Infectious & Tropical Diseases**		
JACS Code:	**A300**	Sponsor License Number:	**EM0DXN1M6**
# Applicant Reference:	**1602857/RITD**	Date of Birth:	**30 December 1980**
Start Date:	**24 April 2016**	End Date:	**24 April 2023**
Fee Status:	**Overseas – Capacity Strengthening**		
Mode of Study:	**Part-Time**	Tuition Fees for 2016/17:	**£2,950**
Supervisor: External supervisor	**Sarah Staedke** **Grant Dorsey**	External Site:	**IDRC Uganda**
Research Department:	**Department of Clinical Research**		

It is recommended that students have a minimum of £12,500 to cover living expenses for a year.

OFFER CONDITIONS: All offer conditions have been met.

[redacted]

James Brown, Head of Registry

31 January 2017

Please tick one box below and sign. You should return your reply to Registry within 28 days.

☑ I accept the offer of a place on the course, subject to the conditions detailed. I accept responsibility for tuition fees and living costs for the whole period of my course. I understand none of the costs of my course can be paid by the School. The School is unable to provide assistance for grants, loans or reductions of fees.

OR

☐ I am unable to accept this offer, and withdraw my application to the School.

Signed: [redacted] Date: 31/Jan/2017

Abel KAKURU **1602857/RITD**

Passport Number: B0798144 Country of Birth: Uganda

PLEASE COMPLETE AND RETURN TO THE REGISTRY WITHIN 28 DAYS OF THE DATE ON THIS OFFER OF ADMISSION

The Registry, London School of Hygiene & Tropical Medicine, Keppel Street, London, WC1E 7HT
E-mail: registry@lshtm.ac.uk **Tel.: +44 (0)20 7299 4646** **Fax: +44 (0)20 7299 4656**

Appendix B: Letter of support from the PROMOTE Study principal investigator

MAKERERE UNIVERSITY
P.O. Box 7072 Kampala, Uganda
E-mail: dean@chs.mak.ac.ug
Phone: 256 414 530020
Fax: 256 414 530020

COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICINE
OFFICE OF THE DEAN

25th March 2016

The Registry,
London School of Hygiene & Tropical Medicine
Keppel Street,
London WC1E 7HT, United Kingdom

Dear Sir/Madam,

Re: Permission for Dr. Abel Kakuru to do his PhD project nested within the PROMOTE Birth cohort 3 study

This is to inform you that Dr. Abel Kakuru has our permission to do his PhD project nested within our new study PROMOTE Birth cohort 3, a randomized controlled trial of intermittent preventive treatment of malaria with monthly sulfadoxine-pyrimethamine versus monthly dihydroartemisinin-piperaquine in HIV-uninfected pregnant women. I am the principle investigator on this study and Abel is a co-investigator and project manager. His PhD project will look at the impact of *Plasmodium falciparum* single nucleotide polymorphisms related to sulfadoxine pyrimethamine resistance on birth outcomes, evaluate the cost-effectiveness of monthly sulfadoxine pyrimethamine versus monthly dihydroartemisinin-piperaquine for prevention of malaria in pregnancy and assess for the impact of malaria in pregnancy on health outcomes in infants among others.

Abel has our full support to do his PhD project. If you need any more information about Abel and his PhD project, please contact me.

Yours sincerely,

Dr. Moses R. Kamya.
Dean and Professor of Medicine
Executive Director, Infectious Diseases Research Collaboration
Email: mkamya@infocom.co.ug

Appendix C: Letter of support from the PROMOTE Study UCSF principal investigator

UCSF
University of California
San Francisco

Department of Medicine
Division of HIV, Infectious Diseases & Global Medicine
UCSF at Zuckerberg
San Francisco General
995 Potrero Ave.
Building 80, Ward 84
San Francisco, CA 94110
tel: 415.476.4082
www.ucsf.edu
hiv.ucsf.edu

23th October 2018

The Chair, London School of Hygiene & Tropical Medicine Ethics Committee
Keppel Street,
London WC1E 7HT, United Kingdom

Re: Permission for Abel Kakuru to use the PROMOTE II Birth Cohort 3 study data for his PhD Project

Dear Sir/Madam,

This is to confirm that Abel Kakuru has my permission to use the PROMOTE II, Birth cohort 3 study data for his PhD project evaluating the impact of malaria in pregnancy and intermittent preventive treatment of malaria in pregnancy (IPTp) on the risk of malaria during infancy.

I am one of the principal investigators of this trial which is a double blind randomized controlled trial of IPTp with monthly sulfadoxine-pyrimethamine versus monthly dihydroartemisinin-piperaquine among HIV-uninfected pregnant women and their infants. Abel is a co-investigator on this trial and the project manager of the study site. He was actively involved in all processes of the study including; project conception, designing the study, protocol writing, obtaining local IRB approvals, development of data collection tools, data collection, and preparing project progress reports among others.

I therefore give Abel my permission to use our data for his PhD. For more information about Abel and his PhD project, please contact me using the email address below.

Yours sincerely,

[redacted]

Prof Grant Dorsey, MD, PhD
University of California San Francisco,

Email: grant.dorsey@ucsf.edu

Appendix D: LSHTM Ethics approval

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Observational / Interventions Research Ethics Committee

Dr Abel Kakuru
LSHTM

6 November 2018

Dear Abel,

Study Title: Impact of malaria in pregnancy and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infants

LSHTM Ethics Ref: 15294

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Protocol / Proposal	Placental Histopathology CRF FINAL	12/11/2014	1.0
Protocol / Proposal	Enrollment CRF FINAL	09/03/2016	1.0
Protocol / Proposal	Mothers Clinic Visit CRF FINAL	09/03/2016	1.0
Protocol / Proposal	Mothers Delivery CRF FINAL	09/03/2016	1.0
Protocol / Proposal	Childs Delivery CRF FINAL	09/03/2016	1.0
Protocol / Proposal	Childs Clinic Visit CRF FINAL	09/03/2016	1.0
Protocol / Proposal	Hospital Admission CRF FINAL	09/03/2016	1.0
Protocol / Proposal	Subject Death CRF FINAL	09/03/2016	1.0
Protocol / Proposal	Subject Withdrawal or Study Completion CRF FINAL	09/03/2016	1.0
Consent form	English future use of bio specimens ICF	08/04/2016	1.0
Consent form	English main ICF_Aug 5, 2016	05/08/2016	1.0
Protocol / Proposal	Main study protocol version 5.0_27 Feb 2018	27/02/2018	5.0
Local Approval	SBSREC Approval Vs 5.0	16/03/2018	5.0
Local Approval	SBSREC Approval Vs 5.0	16/03/2018	5.0
Local Approval	UCSF Approval Vs 5.0 approval	04/05/2018	5.0
Local Approval	UCSF Approval Vs 5.0 approval	04/05/2018	5.0
Investigator CV	Abel Kakuru_CV	20/07/2018	1.0
Protocol / Proposal	Impact of MiP and IPTp on infancy malaria_protocol_version 1.0	30/08/2018	1.0
Covering Letter	Responses to LSHTM Ethics comments	18/09/2018	1.0
Covering Letter	Permission letter-MK	22/10/2018	1.0
Covering Letter	Cover letter-LSHTM ethics application	23/10/2018	1.0

Covering Letter	Permission letter-GD	23/10/2018	1.0

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

Appendix E: Makerere University School of Biomedical Sciences Research Ethics committee initial approval

MAKERERE UNIVERSITY
P.O. Box 7072 Kampala, Uganda
E-mail: irbbiomedicalsciences@gmail.com
irbbiomedicalsciences@chs.mak.ac.ug
Tel:+ 256 772426681
Fax: 256 414 532204

COLLEGE OF HEALTH SCIENCES
SCHOOL OF BIOMEDICAL SCIENCES
HIGHER DEGREES RESEARCH AND ETHICS COMMITTEE

13th May 2016

File: SBS 342

To: Prof. Moses Kamya
Principal Investigator
Department of Medicine
Makerere University

Category of review
[X] Initial review
[ ] Continuing review
[ ] Amendment
[ ] Termination of study
[ ] SAEs

Decision of the School of Biomedical Sciences Higher Degrees Research and Ethics Committee (SBS-HDREC) following its 55th REC meeting held on 3rd March 2016.

In the matter concerning the review of a research proposal entitled **"Prevention of malaria in HIV infected pregnant women and infants"**

The investigator has met all the requirements as stated by **SBS-HDREC** and therefore, the protocol is **APPROVED.**

The approval granted includes all materials submitted by the investigator for **SBS-HDREC** review unless otherwise stated; and is valid until **2nd March 2017**

Any problems of a serious nature related to the execution of the research protocol should be promptly reported to the **SBS-HDREC**, and any changes to the research protocol should not be implemented without approval from **SBS-HDREC**, except when necessary to eliminate apparent immediate hazards to the research participant(s)

Please note that the annual report and the request for renewal where applicable should be submitted to the **SBS-HDREC** office at least six (6) weeks before expiry date of approval.

You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

Signed [redacted]

02 MAR 2017

Dr. Erisa Mwaka [redacted]
Chair person, School of Biomedical Sciences Higher Degrees Research and Ethics Committee

Appendix F: University of California Research Ethics committee initial approval

University of California
San Francisco

Human Research Protection Program
Institutional Review Board (IRB)

Full Committee Approval

Principal Investigator
Dr. Matthew G Dorsey, MD

Co-Principal Investigator

Type of Submission: Initial Review Submission Packet
Study Title: Prevention of Malaria in HIV-uninfected Pregnant Women and Infants

IRB #: 16-18679
Reference #: 157063

Reviewing Committee: San Francisco General Hospital Panel

Study Risk Assignment: Greater than minimal

Approval Date: 04/22/2016 **Expiration Date:** 04/21/2017

Regulatory Determinations Pertaining to This Approval:

This research satisfies the following condition(s) for the involvement of children:
45 CFR 46.405, 21 CFR 50.52: Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects.
Because the adolescents being enrolled in this study are legally entitled to consent to the treatments and procedures involved in the study, Subpart D of 45 CFR 46 does not apply. Parental consent is not required.

Parental Permission and Assent:
Both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.

The research meets all of the conditions of 45 CFR 46.204 for the involvement of pregnant women or fetuses.
The research meets conditions of 45 CFR 46.205 for the involvement of neonates.

This research is not subject to HIPAA rules.

All changes to a study must receive UCSF IRB approval before they are implemented. Follow the modification request instructions. The only exception to the requirement for prior UCSF IRB review and approval is when the changes are necessary to eliminate apparent immediate hazards to the subject (45 CFR 46.103.b.4, 21 CFR 56.108.a). In such cases, report the actions taken by following these instructions.

Expiration Notice: The iRIS system will generate an email notification eight weeks prior to the expiration of this study's approval. However, it is your responsibility to ensure that an application for continuing review approval has been submitted by the required time. In addition, you are required to submit a study closeout report at the

completion of the project.

Documents Reviewed and Approved with this Submission (includes all versions – final approved versions are labeled 'Approved' in the Outcome column):

Consent Documents

Study Consent Form			
Title	Version #	Version Date	Outcome
PROMOTE II BC 3 Future Use of Specimens Informed Consent	Version 1.3	04/08/2016	Approved
PROMOTE II BC 3 Future Use of Specimens Informed Consent	Version 1.0	01/26/2016	
PROMOTE II BC 3 Main Study Informed Consent	Version 1.2	04/08/2016	Approved
PROMOTE II BC 3 Main Study Informed Consent	Version 1.0	01/26/2016	

Other Study Documents

Study Document			
Title	Version #	Version Date	Outcome
PROMOTE II Birth Cohort 3 protocol version 1.1 FINAL	Version 1.1	04/08/2016	Approved
PROMOTE II Birth Cohort 3 protocol version 1.0 FINAL	Version 1.0	01/26/2016	
Birth Cohort 3 protocol full references	Version 1.0	01/20/2016	Approved
Household survey	Version 1.0	01/29/2016	Approved
PROMOTE II DSMB Meeting	Version 1.0	01/26/2016	Approved
PROMOTE II Birth Cohort 3 recruitment sheet	Version 1.0	01/26/2016	Approved
Drugs with known risk	Version 1.0	01/26/2016	Approved
Schedule of routine assessments in infants	Version 1.0	01/26/2016	Approved
Schedule of routine assessments in pregnant women	Version 1.0	01/26/2016	Approved

For a list of all currently approved documents, follow these steps: Go to My Studies and open the study – Click on Informed Consent to obtain a list of approved consent documents and Other Study Documents for a list of other approved documents.

San Francisco Veterans Affairs Medical Center (SFVAMC): If the SFVAMC is engaged in this research, you must secure approval of the VA Research & Development Committee in addition to UCSF IRB approval and follow all applicable VA and other federal requirements. The IRB website has more information.

Appendix G: Makerere University School of Biomedical Sciences Research and Ethics Committee approval for protocol version 5.0

MAKERERE UNIVERSITY
P.O. Box 7072 Kampala, Uganda
E-mail: biomedicalresearch62@gmail.com
Tel:+ 256 752575050
Fax: 256 414 532204

COLLEGE OF HEALTH SCIENCES
SCHOOL OF BIOMEDICAL SCIENCES
HIGHER DEGREES RESEARCH AND ETHICS COMMITTEE

16th March 2018

File: SBS 342

To: Prof. Moses Kamya
Principal Investigator
School of Medicine
Makerere University College of Health Sciences

Category of review
[ ] Initial review
[ ] Continuing review
[X] Amendment
[ ] Termination of study
[ ] SAEs

Re: Approval of an amendment to a research proposal entitled **"Prevention of malaria in HIV infected pregnant women and infants" version 5.0**

Your proposal entitled **"Prevention of malaria in HIV infected pregnant women and infants" version 5.0** was initially reviewed and approved by School of Biomedical Sciences Higher Degrees Research and Ethics Committee (SBS-HDREC) in a letter dated 13th May 2016.

On 6th March 2018, you requested for permission to make the following modifications;

1. To add additional testing for experimental magnetic levitation-based parasite with residual blood.
2. To add a statement under the routine assessment section that in the subset of visits, less than 10μL of blood from finger prick or phlebotomy will be used for magnetic levitation based detection of malaria parasites.
3. To indicate that samples undergoing parasite detection by magnetic levitation, less than 10μL of blood mixed with paramagnetic agent such as gadolinium, placed in close proximity to the earth magnets, and visualized by microscopy and/or a magnified imaging system.
4. To update the version number to version 5.0

The committee considered these amendments and found them appropriate since they will help to validate the results of the magnetic levitation-based parasite detection method in terms of its functionality, usability, efficacy and accuracy.

On behalf of the committee, am glad to inform you that these amendments have been approved.

Final approval should be sought from Uganda National Council for Science and Technology.

Signed.....

MAKERERE UNIVERSITY
SCHOOL OF BIOMEDICAL SCIENCES
16 MAR 2018
RESEARCH ETHICS COMMITTEE
P.O.BOX 7072, KAMPALA

Dr. Erisa Mwaka
Chairperson, School of Biomedical Sciences Higher Degrees Research and Ethics Committee

Appendix H: University of California Research Ethics committee approval for protocol version 5.0

University of California
San Francisco

Human Research Protection Program
Institutional Review Board (IRB)

Full Committee Approval

Principal Investigator
Matthew Dorsey, MD, PhD

Type of Submission: Continuing Review Submission Form
Study Title: Prevention of Malaria in HIV-uninfected Pregnant Women and Infants

IRB #: 16-18679
Reference #: 214928
Reviewing Committee: San Francisco General Hospital Panel
Study Risk Assignment: Greater than minimal

Approval Date: 04/05/2018 **Expiration Date:** 04/04/2019

Regulatory Determinations Pertaining to This Approval:

This research satisfies the following condition(s) for the involvement of children:
45 CFR 46.405, 21 CFR 50.52: Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects.
Because the adolescents being enrolled in this study are legally entitled to consent to the treatments and procedures involved in the study, Subpart D of 45 CFR 46 does not apply. Parental consent is not required.

Parental Permission and Assent:
Both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.

The research meets all of the conditions of 45 CFR 46.204 for the involvement of pregnant women or fetuses.
The research meets conditions of 45 CFR 46.205 for the involvement of neonates.

This research is not subject to HIPAA rules.

All changes to a study must receive UCSF IRB approval before they are implemented. Follow the modification request instructions. The only exception to the requirement for prior UCSF IRB review and approval is when the changes are necessary to eliminate apparent immediate hazards to the subject (45 CFR 46.103.b.4, 21 CFR 56.108.a). In such cases, report the actions taken by following these instructions.

Expiration Notice: The iRIS system will generate an email notification eight weeks prior to the expiration

of this study's approval. However, it is your responsibility to ensure that an application for continuing review approval has been submitted by the required time. In addition, you are required to submit a study closeout report at the completion of the project.

Documents Reviewed and Approved with this Submission:

Other Study Documents

Study Document Title	Version #	Version Date	Outcome
PROMOTE II Birth Cohort 3 protocol version 1.3	Version 1.6	02/27/2018	Approved

For a list of all currently approved documents, follow these steps: Go to My Studies and open the study – Click on Informed Consent to obtain a list of approved consent documents and Other Study Documents for a list of other approved documents.

San Francisco Veterans Affairs Medical Center (SFVAMC): If the SFVAMC is engaged in this research, you must secure approval of the VA Research & Development Committee in addition to UCSF IRB approval and follow all applicable VA and other federal requirements. The IRB website has more information.